Reprinted from the JOURNAL OF DAIRY SCIENCE, Vol. 54, No. 9, Sept. 1971, Pages: 1309–1340

SYMPOSIUM: BOVINE IMMUNE SYSTEM

Introduction

Increasing numbers of scientists are involved in studying the immune system of cattle. In recognition of this development, a symposium on the subject was convened at College Park, Maryland, November 18 to 20, 1970. The symposium was sponsored by the Divisions of Eastern Marketing and Nutrition Research and Veterinary Sciences Research of the Agricultural Research Service, U.S. Department of Agriculture, and the New York State Veterinary College.

The success of the symposium was ensured by contributions from the Veterinary Biologics Division and the Animal Health Division of the Agricultural Research Service, U.S. Department of Agriculture; Miles Laboratories, Inc.; Jensen-Salsbery Laboratories; Fort Dodge Laboratories; Pitman-Moore, Inc.; Norden Laboratories; Armour Pharmaceutical Company; and Elanco Products Company (Eli Lilly).

The objectives of the symposium were threefold: to provide a candid exchange of current information among those studying the bovine immune system; to reach agreement on nomenclature to be applied to bovine immunoglobulins; and to determine and emphasize areas requiring attention and future research effort.

Synopsis

An IgA immunoglobulin class has been established and characterized in cattle (Mach, Butler and Vaerman). The distribution of this protein in the various secretions and the demonstration that it is the principal immunoglobulin synthesized by the exocrine glands, the gut and the respiratory tract (Butler, Mach) is consistent with the occurrence of IgA in other species. The homology of bovine IgA with that of other species has been demonstrated by the detection of common antigenic determinants on human and bovine IgA (Vaerman and Mach) and by the binding of free secretory component (FSC) with dimeric IgA, not only from cattle but from 8 other species (Mach).

Progress toward resolving the heterogeneity of bovine IgG has been aided by the identification of the A1/A2 allotypes on the Fc portion of IgG2 (Blakeslee). The absence of these markers on IgG1 is consistent with its subclass designation. The existence of additional IgG subclasses has been suggested by the ion-exchange and immunoelectrophoretic data of several investigators. However, the designation of bovine subclasses should depend on the demonstration of unique antigenic determinants on the γ chains (Kehoe).

The exact physical dimensions and configuration of the IgG proteins are still in question, although some progress was reported (Butler). A molecular weight of 165,000 for IgG1 has been reported by numerous investigators, although only 150,000 Daltons can be accounted for in heavy and light chains after succinylation, reduction and alkylation (Bezkorovainy). Since it is known that IgG1 is selectively transported into milk, a transport component with a molecular weight of 15,000 Daltons has been postulated (Bezkorovainy).

The question of transport versus local synthesis of the immunoglobulins in the lacteal secretions is not conclusively settled. Local synthesis of IgA in the bovine mammary tissue is low compared to that of non-ruminants (Butler, Mach), although synthesis of FSC (glycoprotein-a) is abundant (Butler, Mach). Mammary tissues showed mainly IgG cells although cells producing other immunoglobulins are present in small numbers (Yurchak). Much more striking immunofluorescence for IgG and glycoprotein-a was noted in the lumen of the

---

1 Senior authorship not implied.

2 Organization and Program Committee.

lacteal ducts than was seen either intercellularly or within the epithelial cells (Yurchak). Quantitative studies of immunoglobulin levels at parturition (Kiddy) and studies of experimentally induced colostrum formation (Smith) confirm the transport of IgG1 from serum to lacteal secretions. The control of such transport may be regulated by the levels of estrogen and progesterone (Smith). The source of IgG2 in the secretions during late gestation and in mastitis is unexplained (Kiddy); however, distention of the udder during these times could cause increased vascular permeability allowing other blood proteins to enter the milk (Campbell). The reverse transfer occurring at the same time could explain the presence of secretory IgA in the serum, particularly before parturition (Mach), and the negative correlation between serum IgA and IgG levels associated with calving (Kiddy).

Another issue in cattle involving transport of immunoglobulin concerns the absorption by the calf gut of maternal immunoglobulins from colostrum. Permeability does not appear to last much beyond one day, and 25% of the calves in one study absorbed few or no immunoglobulins despite normal levels of immunoglobulins in the colostrum (Staley). A tubular system in the apical cytoplasm appears to be involved in the absorption mechanism (Staley).

The development of bovine lymphoid tissue and immunoglobulin producing cells follows the same sequence of appearance as has been reported for the human (Schultz). The first lymphoid tissue is seen in the thymus at 42 days and the first IgM containing cells appear at 59 days. Immunoglobulin G containing cells do not appear until 145 days and IgA cells were not observed even in older fetuses (Schultz). The absence of IgA synthesis in cultures of gut tissue from young calves is consistent with the latter, although IgA synthesis did occur in other secretory tissue (Butler). The delayed development of the secretory immune system in the gut of calves may be related to the absorption of colostral immunoglobulins and to the incidence of intestinal infections. Synthesis and localization of all classes of immunoglobulins in various body organs is comparable to that observed in other mammals (Butler, Yurchak, Mach), with no marked differences observed between the synthesis of IgG1 and IgG2 (Butler). The strong synthesis of IgA by the thymus was unexpected (Butler) but is supported by immunofluorescence in Hassell's corpuscles (Yurchak). Abundant synthesis of IgA in the lacrimal gland, respiratory tract, and all cultures of adult gut tissue (Butler, Mach) were paralleled by very high levels of IgA in tears (Butler, Mach), nasal secretions (Butler, Duncan, Mach), and in intestinal extracts (Mach). Anti-IgA fluorescence is prominent in the lamina propria of the intestine, between the epithelial cells and in the lumen of the crypts much as it has been found in other species (Yurchak). IgG also occurs interstitially but rarely in the lumen and fewer IgG than IgA producing cells are found in the lamina propria (Yurchak). Brilliant anti-glycoprotein-a fluorescence is found intercellularly, in the lumen and within the epithelial cells (Yurchak).

The concentration of the various immunoglobulins in body fluids of the cow is consistent with the results of synthetic and fluorescent studies. The principal immunoglobulins in the serum are IgG1 and IgG2, with the concentration of the former greater than the latter (Mach, Moll) except immediately before parturition (Kiddy). The IgG1 concentration nearly equals the total immunoglobulin (Mach, Kiddy) and total protein (Kiddy) concentration in precolostral and colostral lacteal samples. The predominant immunoglobulin in the lacteal secretions (IgA versus IgG) correlates with the mode of transfer (placental versus colostral) of immunity to the offspring (Mach), and those species studied to date can be grouped into three categories (Vaerman). The ratio of IgG to IgA before and after calving changes significantly (Kiddy). Although no temporal data are yet available, the highest mean values for IgM occur in colostrum (Mach). Variations between individual animals and individual quarters in the udder of the same cow are notable (Kiddy). The problems sometimes encountered with polyvalent IgG antisera (Moll, Kiddy) emphasize the need for monospecific antisera for use in radial immunodiffusion assays.

The kinetics and factors influencing the immune response, as well as the biological activities of different immunoglobulin classes, are generally similar in cattle to those which have been established in other species. Studies on the immune response of cows to various *Brucella abortus* antigens, particularly in regard to whether route of immunization or antigen type was effective in inducing persistent levels of milk antibodies, indicate that persistent low titers are obtainable by repeated udder infusions of low doses of either whole cells or soluble antigens. However, antibodies so induced are of the IgG and IgM classes, rather than the IgA type noted in cows from field cases with

similar persistent milk titers (Jenness). The importance of both quantitative and qualitative measurements of the immune response, especially in attempting to relate immunity induced by vaccination to challenge immunity under field conditions, is emphasized in studies with foot-and-mouth disease virus (Cowan), and *Anaplasma marginale* (Rose). In the viral system electrophoretically fast and slow 7S antibodies react disproportionately in the complement fixation test, while 19S antibody does not fix complement. Quantitative experiments indicate that 25 to 30 molecules of 7S antibody are capable of neutralizing the virus whereas only 2 or 3 19S antibody molecules are required. The radial immunodiffusion technique is particularly applicable for quantitative estimates of both antigens and antibodies (Cowan). A comparison of the complement fixation test with the card agglutination test fails to show a correlation in examination of plasma from animals in the acute stages of anaplasmosis. The agglutination test was found to be useful in detecting antibodies in the plasma of convalescent carriers (Rose). Depletion of circulating lymphocytes in calves results in the suppression of cellular, but not all humoral immune responses (Graber).

A limited quantity of information has been obtained on the sources and protective benefit of antibodies in external secretions. Aerosal exposures of cattle to certain viral (parainfluenza type 3, infectious bovine rhinotracheitis) (Todd) and bacterial (*Pasteurella hemolytica*) (Duncan) agents result in the appearance of antibodies in nasal secretions. A state of protective immunity may be achieved superior to that obtained by parenteral immunization (Duncan). A prerequisite for this may be the ability of the infectious agent to colonize the respiratory tract (Todd). An 11S IgA is the predominant immunoglobulin in nasal secretions prior to and after vaccination procedures. Indirect evidence for its synthesis locally has been achieved in studies on levels of naturally occurring bacterial antibodies in serum and secretions (Duncan).

Vaccination procedures performed locally in the mammary gland (Norcross) and in the genital system of cattle have, thus far, not been notably successful although there is no doubt that local synthesis of immunoglobulin does occur in the mammary gland, vagina and uterus (Butler). Highly effective protection of female cattle toward a localized infection of the genital tract produced by *Vibrio fetus* venerealis can be achieved by parenteral immunization with a whole cell bacterin in complete Freund's adjuvant. Vaccinated heifers achieve high systemic antibody titers of IgM and IgG types. Immunoglobulin G antibodies appear in the cervico-vaginal secretions concurrently, probably by transudation from serum. The IgG subtype which predominates in the uterine, cervical and vaginal fluids has not yet been established. The secretions of naturally infected heifers, in contrast, contain predominantly IgA antibodies which, it may be inferred, are produced locally (Wilkie).

Relatively little research has been performed on allergic diseases which affect cattle. One system which has been well characterized recently is a syndrome in which cows develop an immediate hypersensitivity to their own milk proteins (Campbell). A reagin type antibody is responsible for this condition although it has as yet not been isolated nor characterized as a counterpart of IgE (Campbell). Some progress toward the identification of bovine IgE was reported, however (Yurchak).

The relationship of the immune response to clinical disease has been examined in Johne's Disease, a chronic intestinal infection of cattle produced by *Mycobacterium paratuberculosis* (Aalund). The leukocyte migration reaction has been used in diagnosis, and preliminary results suggest that this test may prove useful in detecting the early, preclinical stages of this disease (Aalund).

The characterization and function of the complement and conglutinin system in cattle are objects of current study. Seasonal fluctuations in serum levels of conglutinin have been observed, as well as marked depressions after calving or abortion and during acute systemic infections (Ingram). The significance of variations in serum conglutinin levels has not been determined.

The findings presented at this conference have served not only to summarize current knowledge, but to point up areas of confusion and ignorance, and thereby bring into focus some of the most urgent areas requiring clarification. Among these may be listed the following:

Heterogeneity of immunoglobulins. A comprehensive definition of bovine immunoglobulin classes, subclasses, L-chain types and subtypes, and allotypes is required. Clarification of additional IgG or IgA subclasses, the characterization of the bovine homocytotropic antibody and the elucidation of other subclass or subtype associated allotypes are areas of immediate concern.

Transport of immunoglobulins. The mechanisms by which IgG1 is transported to the

mammary gland, by which serum derived antibodies enter infected tissues and by which maternal immunoglobulins are absorbed by the calf are not understood. The possible roles of hormonal or other humoral factors in the regulation of these phenomena have been suggested but require further study. The possibility of allogeneic inhibition in maternal-offspring transference has not been investigated. Although chemical differences have been suggested, the structural features which favor IgG1 transport into the lacteal secretions are not resolved. Finally, the issue of local synthesis versus local transport is unresolved for some tissues.

Protective immunity. The protective function of immunoglobulins in external secretions is poorly understood. The role of IgA in conferring protection against bacterial infections has not been defined in any species. A definition of this function is particularly pertinent in the calf gut, as well as in external secretions of mature cattle. Examination of the ratios of IgG1 and IgG2 in either external secretions or serum may elucidate the comparative efficiencies of these immunoglobulins in mediating protection. Some of the principal diseases of cattle involve pathogens in which cellular immunity is an outstanding characteristic. Studies of this phenomenon in cattle and its possible protective function in disease have received virtually no attention (Quinn).

Cattle in basic immunology. Effective use of cattle as tools in the study of basic immune mechanisms needs additional exploration. The role of the secretory component of IgA might best be understood by studying the cow because of the abundance of this protein and its known capacity to bind the IgA of other species. A study of the pronounced selective transport of IgG1 and the absorption of immunoglobulins by the calf gut could lead to an understanding of these phenomena in other species. Finally, the value of cattle as a source of anti-lymphocyte serum and as a model in studying the effect of lymphocyte depletion should be determined.

Proposed Nomenclature for the Immunoglobulins of the Domesticated Bovidae

Cattle (*Bos taurus*)
Sheep (*Ovis aries*)
Goats (*Capra hircus*)

A nomenclature scheme was drawn up by the following investigators who attended the Symposium.

Ole Aalund, Dennis Blakeslee, John E. Butler, J. Robert Duncan, M. James Freeman, Robert Jenness, J. Michael Kehoe, Jean-Pierre Mach, Jan Rapacz, Jean-Pierre Vaerman, and Alex J. Winter.

In addition, we thank T. B. Tomasi, Jr., State University of New York at Buffalo and Alan E. Pierce, Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia, for their suggestions.

Bovidae IgG. The bulk of the immunoglobulins occurring in the serum and lacteal secretions of the three named species possess antigenic determinants (1, 22) and physicochemical characteristics (1, 2, 5, 7, 15, 17, 25, 31) in common with the IgG class as defined in the human (4). Heterogeneity of these immunoglobulins has been demonstrated in all three species (1, 2, 5, 12, 13, 14, 17, 25, 28, 29). Clear-cut evidence for two distinct subclasses is available for all three species (1, 5, 10, 23, 28) although a third has been claimed for the sheep (9). The IgG1 and IgG2 subclasses of cattle will be described.

IgG1. Bovine IgG1 of the serum and colostrum differs from IgG2 in that it migrates faster toward the anode in a basic agar-gel electrophoretic field (1, 10, 24) or slower toward the cathode in acrylamide gel at pH 4.5 (5). It is the predominant immunoglobulin of the lacteal secretions (1, 5, 21, 24) and is antigenically distinct from IgG2. Antisera produced against IgG1 in heterologous species detect antigenic determinants on the γ-chains of IgG1 which are not detected on the γ-chains of IgG2 (23). These differences presumably reside in the Fc fragment in all three species although this has been demonstrated only in the sheep (10).

IgG2. Bovine IgG2 constitutes less than one-half of the total IgG in the serum of most cattle (26), carries a greater net positive charge than IgG1 and differs antigenically from IgG1 in its γ-chains (23). Bovine IgG2 carries the A1/A2 allotypes on its Fc fragment which are absent on the γ-chains of IgG1 (3).

The IgG subclasses described for cattle can be shown to be antigenically homologous to the IgG1 and IgG2 of sheep (10) and presumably of the goat. Additional IgG subclasses would be based upon the demonstration of specific γ-chain antigenic determinants distinct from those of IgG1 and IgG2 and which occur on molecules in all normal members of the species.

Bovidae IgA. Bovine, ovine, and caprine IgA are defined by their cross reactions with anti-human α-chain antisera (21, 33). Bovine IgA has physicochemical (6, 7, 20, 30, 33), distri-

butional (8, 21, 30) and synthetic characteristics (8, 21, 33) similar to those described for human IgA (32) with exception of the mammary gland and its secretions (8, 21). Bovine IgA has been shown to be homologous to that of the goat and sheep (27). Designation of subclasses of this immunoglobulin would be based upon demonstrated antigenic differences shown to reside in the α-chains of molecules which occur in all normal members of the particular species.

Bovidae IgM. Bovine IgM is described by its cross reactions with anti-human μ-chain antisera (22). Ovine and caprine IgM are defined by their cross reactions with anti-bovine μ-chain antisera (27). The IgM described for these species has physicochemical characteristics similar to human IgM (5, 7, 22, 25). Designation of subclasses of this immunoglobulin would be based upon demonstrated antigenic differences shown to reside in μ-chains of molecules which occur in all normal members of the particular species.

Bovidae free secretory component. Bovine glycoprotein-a (11) and free secretory component (18, 20) are similar as defined by their antigenic cross reactivity (19) and by their partial common antigenicity with bovine secretory IgA (6, 7, 20, 21). The demonstration of sequence homology or immunological cross reactions or both between this protein and human secretory component will be necessary to establish whether they are homologous. Bovine glycoprotein-a and human secretory component have identical patterns of immunofluorescence in the intestinal lumen of the respective species (34). Free secretory components of goat and sheep should be shown to be homologous to that of cattle on the basis of antigenic cross reactivity.

Light polypeptide chains. All classes and subclasses of bovine immunoglobulins share common antigenic determinants on their light polypeptide chains (5, 7). The light chains of cattle share antigenic determinants with those of sheep (16) and presumably the goat and are predominantly of the lambda type (16).

References

(1) Aalund, O. 1968. Heterogeneity of Ruminant Immunoglobulins. Thesis, Munksgaard, Copenhagen, Denmark.

(2) Aalund, O., J. W. Osebold, and F. A. Murphy. 1965. Isolation and characterization of ovine gamma globulins. Arch. Biochem. Biophys., 109: 142.

(3) Blakeslee, D., J. Rapacz, and J. E. Butler. 1971. Bovine immunoglobulin allotypes. Symposium: Bovine Immune System. J. Dairy Sci., 54: 1319.

(3a) Blakeslee, D., J. E. Butler, and W. H. Stone. 1971. Immunogenetics of two immunoglobulin allotypes in cattle. J. Immunol., In press.

(4) Bulletin World Health Organization. 1964. Nomenclature for human immunoglobulins, 30: 447. [Also reprinted in Immunochemistry, 1: 145].

(5) Butler, J. E. 1969. Bovine immunoglobulins: A review. J. Dairy Sci., 52: 1895.

(5a) Butler, J. E. 1971. A review of the bovine immunoglobulins. Symposium: Bovine Immune System. J. Dairy Sci., 54: 1315.

(6) Butler, J. E., E. J. Coulson, and M. L. Groves. 1968. Identification of glycoprotein-a as a probable fragment of bovine IgA. Abstr., Federation Proc., 27: 617.

(6a) Butler, J. E., M. L. Groves, and E. J. Coulson. 1970. The identification of a secretory immunoglobulin in the cow that is antigenically related to glycoprotein-a. Abstr., Federation Proc., 29: 642.

(7) Butler. J. E. 1971. Physicochemical and immunochemical studies on bovine IgA and glycoprotein-a. Manuscript submitted.

(8) Butler, J. E., C. F. Maxwell, C. S. Pierce, C. A. Rock, C. A. Kiddy, and R. Asofsky. 1971. Distributional and synthetic studies on bovine IgA. Proc. Soc. Exp. Biol. Med., In press.

(8a) Butler, J. E., C. A. Kiddy, C. F. Maxwell, M. B. Hylton, and R. Asofsky. 1971. Synthesis of immunoglobulins by various tissues of the cow. Symposium: Bovine Immune System. J. Dairy Sci., 54: 1323.

(8b) Butler, J. E., C. A. Kiddy, C. S. Pierce, and C. A. Rock. 1971. Quantitative changes associated with calving in the levels of bovine immunoglobulins in selected body fluids. I. Changes in the levels of IgA, IgG1 and total protein. Manuscript submitted.

(9) Curtain, C. C. 1969. A new immunoglobulin subclass in sheep. Immunology, 16: 373.

(10) Feinstein, A., and M. J. Hobart. 1969. Structural relationship and complement fixing activity of sheep and other ruminant immunoglobulin G subclasses. Nature, 223: 950.

(11) Groves, M. L., and W. G. Gordon. 1967. Isolation of a new glycoprotein-a and a γG-globulin from individual cow milks. Biochemistry, 6: 2388.

(12) Harrison, E. T., and M. G. Madge. 1967. Isolation and characterization of sheep γ1- and γ2-immunoglobulins and their polypeptide chains. Biochim. Biophys. Acta, 147: 52.

(13) Heimer, R., D. W. Jones, and P. H. Maurer. 1969. The immunoglobulins of sheep colostrum. Biochemistry, 8: 3937.

(14) Heimer, R., L. G. Clark, and P. H. Maurer. 1969. Immunoglobulins of sheep. Arch. Biochem. Biophys., 131: 9.

(15) Hess, E. L., and H. F. Deutsch. 1948. Biophysical studies of blood plasma proteins. VIII. Separations and properties of the γ-globulins of the sera of normal cows. J. Amer. Chem. Soc., 70: 84.

(16) Hood, L., W. R. Gray, B. G. Sanders, and W. J. Dreyer. 1967. Light polypeptide chain evolution. Cold Spring Harbor Symp., 32: 133.

(17) Kickhofen, B., D. K. Hammer, and D. Schell. 1968. Isolation and characterization of γG type immunoglobulins from bovine serum and colostrum. Hoppe-Seyler's Z. Physiol. Chem., 349: 1755.

(18) Mach, J. P. 1971. *In vitro* combination of human and bovine free secretory component with IgA of various species. Nature, 228: 1278.

(19) Mach, J. P., and J. E. Butler. 1971. Antigenic identity of bovine free secretory component and glycoprotein-a. Personal communication.

(20) Mach, J. P., J. J. Pahud, and H. Isliker. 1969. IgA with "secretory piece" in bovine colostrum and saliva. Nature, 223: 952.

(21) Mach, J. P., and J. J. Pahud. 1971. Secretory IgA, a major immunoglobulin in most bovine external secretions. J. Immunol., 106: 552.

(21a) Mach, J. P., and J. J. Pahud. 1971. The bovine secretory immune system. Symposium: Bovine Immune System. J. Dairy Sci., 54: 1327.

(22) Mehta, P. D., and T. B. Tomasi, Jr. 1969. Comparative studies of mammalian immunoglobulins. Abstr., Federation Proc., 28: 820.

(23) Milstein, C. P., and A. Feinstein. 1968. Comparative studies of two types of bovine immunoglobulin G heavy chains. Biochem. J., 107: 559.

(24) Murphy, F. A., O. Aalund, J. W. Osebold, and E. J. Carroll. 1964. Gamma globulins of bovine lacteal secretions. Arch. Biochem. Biophys., 108: 230.

(25) Murphy, F. A., J. W. Osebold, and O. Aalund. 1965. Physical heterogeneity of bovine γM and γG globulins. Arch. Biochem. Biophys., 112: 126.

(26) Nansen, P. 1970. Metabolism of bovine immunoglobulin-G. Thesis, Munksgaard, Copenhagen, Denmark.

(27) Pahud, J. J., and J. P. Mach. 1970. Identification of secretory IgA, free secretory piece and serum IgA in the ovine and caprine species. Immunochemistry, 7: 679.

(28) Pan, I. C., A. M. Kaplan, R. L. Morter, and M. J. Freeman. 1968. Spectrum of ovine immunoglobulins. Proc. Soc. Exp. Biol. Med., 129: 867.

(29) Pierce, A. E., and A. Feinstein. 1965. Biophysical and immunological studies on bovine immune globulins with evidence for selective transport within the mammary gland from maternal plasma to colostrum. Immunology, 8: 106.

(30) Porter, P., and D. E. Noakes. 1970. Immunoglobulin IgA in bovine serum and external secretions. Biochim. Biophys. Acta, 214: 107.

(31) Smith, E. L. 1946. The immune proteins of bovine colostrum and plasma. J. Biol. Chem., 164: 345.

(32) Tomasi, T. B., Jr., and J. Bienenstock. 1968. Secretory immunoglobulins. Adv. Immunol., 9: 2.

(33) Vaerman, J. P. 1970. Studies on IgA immunoglobulins in man and animals. Thesis, Sintal-Louvain, Belgium.

(34) Yurchak, A. M., J. E. Butler, and T. B. Tomasi, Jr. 1971. Fluorescent localization of immunoglobulins in the tissues of the cow. Symposium: Bovine Immune System. J. Dairy Sci., 54: 1324.

SESSION I. CHARACTERISTICS OF BOVINE IMMUNOGLOBULINS AND RELATED MOLECULES

Chairman: Robert Jenness, University of Minnesota

Review of the Bovine Immunoglobulins

JOHN E. BUTLER
Eastern Marketing and Nutrition Research Division
ARS, USDA, Washington, D.C. 20250

Three antigenically distinct classes of immunoglobulins, IgG, IgA, and IgM have been identified in cattle. Two subclasses of IgG immunoglobulins are currently recognized: IgG1 and IgG2. IgG1 demonstrates considerable charge heterogeneity and is normally the most abundant immunoglobulin in the serum and lacteal secretion of the cow. Cow IgG1 accumulates to very high levels in colostral and precolostral lacteal secretions by a mechanism which selectively transports it from the blood. IgG1 is apparently the same as the γGs described by Kickhofen et al. (16). IgG2 normally makes up less than half of the total serum IgG immunoglobulins and carries the A1/A2 allotypic markers. The most typical form for bovine IgA is an 11 S protein containing a secretory component although both physicochemical and antigenic heterogeneity can be demonstrated. IgA is the principal immunoglobulin in and synthesized by the exocrine glands, the gut and the respiratory tract. The only exceptions are the mammary gland and the reproductive tract where IgG production may equal or be more abundant than IgA production. Free secretory component (glycoprotein-a) can be most easily demonstrated in normal milk. Serum IgA occurs in small amounts apparently as a dimer. The nature and distribution of bovine IgM is similar to its homolog in other species. Like their homologs in others species, all bovine immunoglobulins share common light polypeptide chain determinants and can be prepared by classical physicochemical methods. Physicochemical and immunochemical characteristics of each protein can be demonstrated by disc electrophoresis, ion-exchange and gel filtration chromatography, ultracentrifugation and immunoelectrophoresis (Table 1). After disc electrophoresis at pH 4.5, in gels with or without urea, IgM remains at the stacking gel-separating gel interface while IgA moves one-fourth of the distance down the gel and appears as one or two prominent zones. IgG1 from serum or colostrum and IgG2 move half the length of the gel with IgG2 moving slightly faster. Using standard DEAE chromatographic methods, IgG2 is eluted in the fall-through peak whereas IgG1 is eluted over a broad range with a principal peak at 0.15 M NaCl, pH 8.3. Secretory IgA and IgM are eluted sequentially after IgG1.

Antibody activity has been demonstrated in all classes and subclasses of bovine immunoglobulins. IgG1 is a powerful complement fixing antibody in the cow and IgM and IgA are effective agglutinating antibodies. In serum, early antibody response appears in the IgM and IgG2 fractions and shifts to the IgG1 fraction during hyperimmunization. The nature and involvement of the various immunoglobulins in the local immune response of the cow is being studied in various laboratories. Reaginic activity appears most consistently in the IgG1 fraction although the immunoglobulin responsible may be a contaminating IgE class immunoglobulin. Identification of bovine IgE appears imminent. Despite the amount of physiological work done on cattle, detailed investigations into the biological activity, physicochemical characteristics and significance of each bovine immunoglobulin are just beginning. Table 1 is from Butler (6) with new data added.

TABLE 1. Characteristics of bovine immunoglobulins.

Classes and subclasses	IgG1	IgG2	IgM	IgA
Physicochemical characteristics				
Electrophoretic mobility	Fast γ and β_2	γ	β_2	β_2
$S_{20,w}$	6.5–7.0	6.5–7.0	19.5	[1][11[c,d,e](7[e]–15[d])]
Total CHO(%)	2–3	2–3	10–12	6–10[c,d]
Hexose	[1.05[b],1.51[a]]	[0.99[b],1.40[a]]		
Hexosamine	[1.57[b],0.91[a]]	[1.61[b],0.77[a]]		
Fucose	0.12[a]	0.18[a]		
Sialic acid	0.28[b]	0.15[b]		
Molecular weight $\times 10^{-3}$	163[a]	[163[a],150[b]]	900[a], 1030[k]	385[c,d]
H-Chain molecular weight $\times 10^{-3}$	55–58[a,b]	[55–58[a],54[b]]	64–70[a], 76[k]	60–63[a]
Biological characteristics				
Antibody activity	+	+	+	+
Allotype A1 (A2?)[j]	–	+	–	–
Allotype B1[j]	+	+	+	+
Half-life (days)	9.6	17.7		
Placental transmission	–	–	–	–
Lacteal transmission	Pronounced	?	?	?
Reaginic activity	+ (?)	–	–	–
Complement fixation	+	+ (?)	+ (?)	
Concentration (mg/ml)				
Serum	[11.6[g],10.5[f]]	[7.9[f]]	[2.6[i],2.5[f],2.8[h]]	[0.08[g],0.3[f],0.8[e]]
Colostrum	[33.8[g],75.0[f]]	[1.9[f]]	[3.2[i],4.9[f],4.9[h]]	[2.0[g],4.4[f],4.5[e]]
Saliva	[0.04[g],0.03[f]]	[0.01[f]]	[0.01[f]]	[0.23[g],0.56[f]]
Milk	[1.15[g],0.35[f]]	[0.06[f]]	[0.04[f]]	[0.25[g],0.05[f],0.20[e]]
Nasal secretions	[0.04[f]]	[0.02[f]]	[trace]	[1.95[f]]
Tears	[0.52[g],0.30[f]]	[0.12[f]]	[0.06[f]]	[3.8[g],2.6[f]]
Total IgG concentration				
Serum	[22.0[g],13.0[h],26.4[i],18.4[f]]			
Colostrum or precolostrum	[37.4[g],34.0[h],43.3[i],76.9[f]]			

[1] Brackets enclose all data available for the immunoglobulin in question.

[a] Groves et al. (14). [b] Kickhofen et al. (16). [c] Mach et al. (20). [d] Butler et al. (7). [e] Porter and Noakes (29). [f] Mach and Pahud (21). [g] Butler et al. Unpublished data. [h] Penhale and Christie (26). [i] Klaus et al. (17). [j] Blakeslee et al. (4). [k] Mukkur, T. K. S. and A. Froese. 1971. Immunochem. 8:257.

Subclasses of Bovine IgG

J. MICHAEL KEHOE
Mount Sinai School of Medicine
City University of New York, New York 10000

The existence of subclasses of IgG is now firmly established in several species. The characterization of such subclasses is most complete for man where detailed immunologic and structural studies have been facilitated by the availability of the homogeneous proteins produced by many patients with multiple myeloma. Immunoglobulin subclass distinctions within any species have generally been made first on the basis of differing antigenic and electrophoretic properties which in turn reflect differences in heavy chain structure. Different human IgG subclasses also possess different arrangements of the interheavy chain disulphide bonds (28). Amino acid sequences, though not yet complete for all human subclasses, have confirmed that heavy chain sequence differences between IgG subclasses do exist. Functional differences have also been demonstrated and are presumably a consequence of subclass-unique features of covalent structure.

In cattle, the existence of two IgG subclasses is widely accepted, and the presence of a third has been suggested (16). The different subclasses have generally been prepared by ion-exchange chromatography from whole serum, since myeloma proteins have not yet been discovered in this species. Unique subclass properties have been shown by classical immunoelectrophoretic methods, as well as by structural studies of subclass specific heavy chains (22). Functionally, particular interest resides in the selective accumulation of one bovine IgG subclass in colostrum (23). The structural features responsible for this property may well reside in the Fc fragment of molecules of this subclass.

Among the open questions concerning bovine IgG subclasses, the following appear particularly important:

1) Are there more subclasses to be identified?
2) What are the heavy chain amino acid sequence differences responsible for the subclass distinctions?
3) How do the bovine subclasses relate, structurally and functionally, to those of other species?

To verify the existence of subclasses of a given immunoglobulin class in any species, criteria such as the following can be used:

1) Subclass specific antigenic determinants must be located on Fc fragments of test molecules.
2) All subclasses must be present in normal sera of healthy members of the species.
3) Any homogeneous immunoglobulin found must fall into a unique subclass.
4) Different subclasses often mediate different biological activities.
5) Different subclasses will show different heavy chain amino acid sequences.

Identification and characterization of additional subclasses in cattle would, of course, be aided enormously by the availability of pure proteins of a given subclass. The discovery of myeloma paraproteins or the production of highly purified bovine anticarbohydrate antibodies of the type described by Krause (12) in rabbits would fulfill such a need.

Comparison Between Several Mammalian IgAs, Including the Bovine

Jean-Pierre Vaerman
Clinques University
St. Pierre, Louvain, Belgium

To compare various mammalian IgAs to human IgA, it is first necessary to identify IgA unambiguously in these non-human species. The best criterion to establish this identification is the finding of large homologies between the amino acid sequence of the human alpha chain and that of the heavy chain of the animal immunoglobulin proposed as homologous to human IgA. Since this goal is difficult to achieve, another decisive criterion was found in immunological cross-reactions between antisera specific for human IgA and IgA from dogs, cats, cows, goats, sheep, pigs, horses, and hedgehogs, the latter being primitive mammals. Taking advan-

tage of these cross-reactions, antisera were prepared against the IgAs, as well as against other immunoglobulins in the sera of the eight species. The specificity of these antisera was demonstrated by immunoelectrophoresis. These antisera then allowed a study of the behavior of these various immunoglobulins in the sera of the eight species by DEAE-cellulose chromatography, by gel-filtration on Sephadex G-200 and by density gradient ultracentrifugation. The DEAE-chromatographic behavior of these eight different mammalian serum IgAs resembled grossly that of human serum IgA in that all IgAs were eluted after the bulk of the IgGs, and slightly before the IgMs. The molecular size of the major fraction of serum IgA from all these species was compatible with the size of a dimer molecule (9 to 10 S), are estimated by gel-filtration and density gradient ultracentrifugation, in contrast to the human serum IgA which is essentially (± 90%) a 7 S monomer. The molecular size of the major form of IgA present in various exocrine secretions (and especially in colostrum and milk) of these animals was studied in the same way and found to be very similar to that of the main form of human exocrine IgA (± 11 S). The occurrence of IgA_2-like molecules was demonstrated by acid-urea starch-gel-immunoelectrophoresis, in purified unreduced canine, equine, and (probably) porcine milk IgA. The existence of a secretory component was demonstrated immunologically for the canine and porcine species by spurring of exocrine IgA over serum IgA, using antiserum against exocrine IgA. Free secretory component was found in concentrated canine milk whey, urine, saliva, and tears. Concentration ratios, in secretion versus serum, were measured for IgA and other immunoglobulins by single radial immunodiffusion with specific antisera. This ratio was highest for IgA in practically all (53/55) exocrine secretions tested. Insofar as IgA levels in lacteal secretions are concerned, these mammals can be classified into 3 groups: (a) one group where IgA predominates both in colostrum and throughout lactation [human (and primates?)]; (b) a second group (dog, pig, horse) where the predominant immunoglobulin of colostrum is IgG, but, during the first few days of lactation, there is a sharp drop in IgG so that IgA becomes predominant; (c) in a third group (cows, goats and sheep), IgA is a minor immunoglobulin both in colostrum and throughout lactation. The distribution of different classes of immunoglobulin-containing cells, examined by immunofluorescence, disclosed that IgA type cells were consistently abundant and predominant in the intestinal mucosa, occasionally abundant in mesenteric lymph nodes, and consistently scarce in spleen and peripheral lymph nodes.

The fate of the IgA produced by intestinal mucosal plasma cells was investigated in the dog by measuring the concentration ratios of IgA and other proteins in mesenteric lymph versus serum of the same animal, and by comparing the specific radioactivities of serum and mesenteric lymph IgA in a dog who received biosynthetically ^{14}C-labelled plasma IgA. The conclusion was that about 80% of the IgA in mesenteric lymph (whose IgA level exceeded that of serum by a factor of 2 to 18) orginates in the intestinal mucosa and that the latter organ represents the major source of canine serum IgA.

Quaternary Structure of Bovine Colostrum Immunoglobulin

ANATOLY BEZKOROVAINY
Rush-Presbyterian-St. Luke's Medical Center
Chicago, Illinois 60600

Many animal serum proteins concerned with resistance to disease consist of more than one polypeptide chain, and the elucidation of the structure-function relationships in such proteins requires that they be dissociated into their component polypeptide chains. To overcome the disadvantages of classical methods for the dissociation of proteins into subunits, we have developed a procedure involving succinylation of proteins with succinic anhydride, followed by reduction-alkylation with mercaptoethanol and iodoacetate, respectively. Although the latter procedure is performed in the presence of urea, the urea can subsequently be dialyzed out without precipitating the modified protein. As a model protein we used bovine colostrum IgG1, prepared by $(NH_4)_2SO_4$ precipitation, in an electrophoretically and ultracentrifugally homogeneous state. The succinylated-reduced-alkylated protein was resolved into its subunits by

chromatography on Sephadex G-200 columns with dilute aqueous buffer solvents as eluants. A heavy chain fraction, mol wt 50,000, and a light chain fraction, mol wt 25,000, were isolated. Since this accounted for only 150,000 Daltons out of the 165,000 previously determined for the intact protein, the question is raised concerning the possible existence of a small transfer piece in the colostrum IgG1. The disadvantage of using succinylation to break physical interactions in proteins is that the succinylated proteins become devoid of their original antigenic properties. It is impossible to remove the succinyl residues from such proteins without drastic acid hydrolysis. However, substitution of maleic anhydride for succinic anhydride can, in part, overcome this difficulty. Experience with transferrin, an iron-binding protein with antimicrobial properties, showed that maleylation resulted in the same extent of amino group blockage, denaturation, and loss of antigenic properties as succinylation. Yet some 93% of the maleyl groups could be removed from the protein by mild acid hydrolysis at pH 3.5 (citrate buffer) for 5 days at 37 C with the reversal of denaturation. Some 75% of the iron binding activity and full antigenic activity were restored. Maleylation of bovine IgG1, like succinylation, resulted in blocking 75% of the amino groups, decreased sedimentation coefficient from 6.4 to 4.8 (1%), and a loss of antigenic activity. Attempts to remove the maleyl groups under conditions used for transferrin resulted in complete insolubilization of the protein at pH 3.5 to 9. However, mild acid hydrolysis at pH 3.5 for 2 days at 37 C in the presence of 0.1 M propionate resulted in the insolubilization of only 50% of the protein. Analysis of the soluble material showed that all amino groups had been regenerated, that the sedimentation constant had returned to 6.4 S, and that the antigenic properties had been recovered. It is concluded that succinylation and maleylation are useful methods for the elucidation of quaternary structures of immune globulins.

Bovine Immunoglobulin Allotypes

DENNIS BLAKESLEE,[1] JAN RAPACZ,[2] and JOHN E. BUTLER [3]

[1] Department of Pathology, School of Medicine
University of Pennsylvania, Philadelphia 19100
[2] Laboratory of Genetics, University of Wisconsin, Madison 53706
[3] Eastern Marketing and Nutrition Research Division, ARS
USDA, Washington, D.C. 20250

Three antisera detecting different allotypic specificities, designated A1, A2 and B1, of cattle immunoglobulins were produced by isoimmunizations with purified IgG together with Freund's complete adjuvant. Successful immunizations were made both intramuscularly and subcutaneously. The A1 and B1 specificities have been examined immunochemically and all three have been analyzed genetically.

The interchain disulfide bonds of IgG derived from a serum that reacted strongly with both anti-A1 and anti-B1 were cleaved and blocked by oxidative sulfitolysis. The heavy (H) and light (L) chains were separated by gel filtration through Sephadex G-100 equilibrated and eluted with a 5 M urea, 0.05 M formic acid solution. The purified H and L chains were then tested for their ability to react with anti-A1 and anti-B1 and inhibit the precipitation of the antisera with A1(+) or B1(+) IgG in Ouchterlony immunodiffusion. The isolated H chains inhibited anti-A1 but not anti-B1, whereas L chains inhibited anti-B1 only. This demonstrated that the A1 specificity was located on IgG H chains and that B1 was an L chain marker.

Cattle IgM was obtained free of other immunoglobulins by recycling gel filtration on G-200. IgG1, IgG2 and IgA were isolated from serum or colostrum or both by a combination of ion-exchange and gel filtration chromatography and by specific immunoabsorption. Anti-B1 reacted with IgG1, IgG2, IgA and IgM from B1(+) cows. This was expected since it is known that all classes of immunoglobulins possess common light chains. Anti-A1 reacted only with IgG2 molecules derived from A1(+) sera. In contrast to L chains, H chains are specific, each immunoglobulin class or subclass possessing a unique type. These findings on the distribution of the A1 and B1 allotypes among H and L chains are consistent with the general view of immunoglobulin structure worked out in a variety of species. Furthermore, it was

determined that the A1 specificity was located on the c-terminal half or Fc fragment of IgG2 H chains. IgG from an A1(+) B1(+) serum was digested with papain in the presence of cysteine. The resulting Fab and Fc fragments were isolated by DEAE and CM ion-exchange chromatography and gel-filtration. Fc fragment inhibited anti-A1 whereas Fab fragment did not. Anti-B1 was inhibited by Fab fragment, since this portion of the IgG molecule carries an intact L chain.

An analysis of mating data has shown that the A1, A2 and B1 specificities are controlled by 2 loci. The *bva* locus has 2 alleles, bva^1 and bva^2, controlling the allotypic markers A1 and A2, respectively. The A2 specificity has not been examined for its distribution among different immunoglobulin classes or H and L chains. However, since A1 and A2 are controlled by allelic genes, it is virtually certain that A2 is also a marker of the Fc portion of IgG2 molecules. The *bvb* locus, with its single detectable allele, bvb^1, controlling the B1 specificity, appears to be inherited independently of *bva* and is certainly not closely linked to it.

Eleven different breeds were tested for the frequency of bva^1 and bvb^1. In all, the frequency of bva^1 was high (generally .8 or greater), except in the Hereford (about .5). The bvb^1 allele was infrequent or absent in these breeds. Similar data for bva^2 are being collected.

New antisera detecting allotypic specificities of immunoglobulins have been produced and are now being studied for their relationship to A1, A2, and B1.

Complement and Conglutinin in the Cow

D. G. INGRAM
Ontario Veterinary College
Guelph, Ontario, Canada

Complement is a group of globulins which exist in serum in an inactive form. They may be considered to be complex proenzymes which require sequential activation. The complement system comprises 9 distinct recognizable components designated C1, C2, C3, C4, C5, C6, C7, C8, and C9.

Cell lysis by complement usually requires all 9 components and is considered the prototype of complement activity. In the hemolytic system of sheep red blood cells sensitized with rabbit hemolysin, one molecule of IgM antibody or two closely associated IgG molecules combined with antigen on the cell surface form a site at which the complement system can be activated. The components react in the sequence C1, C4, C2, C3, C5, C6, C7, C8, and C9 to produce lesions or "holes" on the cell membrane which are 80 to 100 Å in diameter. Lysis of the cells begins with the loss of intracellular small molecules such as potassium and the subsequent influx of extracellular water from osmotic pressure results in swelling and eventual rupture of the cell.

A number of the biological effects of complement do not involve all 9 components. The cell bound intermediate C1,4 will react with some immunoconglutinins. The complex C1, 4,2,3 is required for immune adherence, conglutination, complement-dependent opsonization and adherence to leucocytes. C1,4,2,3,5,6 is involved in the Arthus reaction. During activation of the complement components some of the proteins are cleaved to produce fragments some of which have recognized biological activities: C3a and C5a have chemotactic and anaphylatoxic activities, C(567)a has chemotactic activity. These examples of complement activities indicate the complexities of this system.

The complement activity in bovine serum has not been studied in detail. It is known that bovine complement has relatively low hemolytic activity when measured by the usual system of sheep red blood cells sensitized with rabbit antibody. However, bovine complement has good hemolytic activity for guinea pig red blood cells. Bovine serum is also a good source of conglutinating complement.

Conglutinin is a naturally occurring serum protein of cattle and certain other species. Conglutinin can be detected in bovine fetal serum as early as the 110th day of gestation and the amount increases gradually until birth.

In adult cows the amount of conglutinin drops sharply at calving time to a low point at about 2 weeks after parturition. Then the titer increases to normal adult levels over 4 to

5 months. A seasonal fluctuation in conglutinin titers occurs with lowest levels in late spring and peak activity in late fall.

Mild or local infections do not appear to alter the conglutinin level in serum but acute systemic infections cause a significant decrease in serum titer of conglutinin. Three non-infectious abortions caused a drop in conglutinin levels similar to that which occurs at the time of normal calving.

Conglutinin is a serum globulin which reacts with C1,4,2,3 and can fix complement but is structurally and antigenically distinct from immunoglobulins.

SESSION II. TRANSPORT, DISTRIBUTION AND SYNTHESIS OF BOVINE IMMUNOGLOBULINS

Chairman: Arthur M. Silverstein, Johns Hopkins University, Baltimore, Maryland

Ontogeny of the Bovine Immune Response

R. D. SCHULTZ, H. W. DUNNE, and C. E. HEIST
Pennsylvania State University, University Park 16802

Knowledge of the time at which immunologic competence develops in the fetus and the cells and organs concerned with that development is of significance in the basic understanding of the immune response.

This study, unlike others, considered the effect of natural antigenic stimulation on the development of the bovine immune response. Three embryos and 108 fetuses were evaluated for lymphoid tissue development, immunoglobulin containing cells, and serum immunoglobulins. The methods of assay included immunofluorescence, radial diffusion, electrophoresis, and special staining techniques. Possible sources of antigenic stimuli to the fetuses were determined by standard bacteriologic, virologic, and serologic methods.

The thymus, the first lymphoid organ identified, was present at 42 days of gestation. Lymphocytes were identified in the peripheral blood at 45 days. The spleen, structurally present at 55 days, contained red and white pulp which was differentiated in certain fetuses between 80 and 100 days of age. The peripheral lymph nodes, first identified at 60 days of gestation, were primarily composed of reticular and mesenchymal cells. Mesenteric lymph nodes were identified at 100 days of gestation. Additional lymphoid tissue of the G-I tract including the Peyer's patches, was observed in a bacterial infected 175 day fetus. Immunoglobulin (Ig) M containing cells were first identified, by immunofluorescence, in a single fetus at 59 days of gestation. IgG containing cells were first identified at 145 days of gestation in a bacterial and viral infected fetus. IgM producing cells were in 36 fetuses and IgM and IgG cells in 7 fetuses. Immunoglobulin containing cells were found most frequently in the spleen and lymph nodes. However, they were also found in the thymus, tonsil, and bone marrow of some fetuses. Immunoglobulin containing cells were not identified in the hemal nodes, blood "buffy coat", Peyer's patches, heart or lungs of certain fetuses with positive cells in the spleen or lymph nodes or in both.

IgM was not identified in the serum until 130 days of gestation and IgG at 145 days of gestation. Immunoglobulin was present in 39 fetal serum samples.

Assuming the Ig containing cells and the Ig in the serum was specific antibody that resulted from antigen stimulation, fetuses were examined for bacterial, viral, and maternal antigens. IBR viral antigens were present in three fetuses and BVD antigens in one fetus. However, viruses were not isolated in primary embryonic bovine kidney cells. Two of the three fetuses with IBR antigens had neutralizing antibody to the virus. Bacteria, including *Escherichia coli, Mima polymorpha* var. *oxidans* and a *Lactobacillus sp* were isolated from four fetuses. Serum samples from 8 of 20 fetuses, with immunoglobulin in the serum, contained antibodies which agglutinated maternal red blood cells. These antibodies were heat stable (56 C, 36 min) and were sensitive to 0.1 M 2-ME.

Ten fetuses, over 220 days of age, with IgM

and IgG containing cells in the spleen were examined for IgA containing cells. Antiserum to IgA and glycoprotein-a (obtained from John E. Butler, USDA, Washington, D.C.) was conjugated and reacted with spleen, lymph nodes, thymus, intestinal tissue, particularly of the lower ileum, and lacrimal gland tissue of 3 fetuses. IgA containing cells were not found in any of the tissues. Serum samples from the group of fetuses with IgM and IgG were reacted in Ouchterlony double diffusion test with IgA and glycoprotein-a antisera. All serum samples were negative.

Table 1 is a summary of lymphoid tissue development and the appearance of immunoglobulins in the bovine fetus.

The results of the time at which lymphoid tissue developed, immunoglobulins appeared and the sequence of immunoglobulin development in the bovine fetus were similar to results reported for immunologic development of the human fetus.

TABLE 1. Lymphoid tissue development in the bovine fetus.

Tissue	Time of appearance (days)
Thymus	42
Blood lymphocytes	45
Spleen	55
IgM containing cells	59
Peripheral lymph nodes	60
Mesenteric lymph nodes	100
Serum IgM	130
Blood granulocytes	130
IgG containing cells	145
Serum IgG	145
Anti-maternal RBC Ab.	155
Anti-IBR Ab.	165
Lymphoid tissue (G-I tract)[a]	175

[a] Does not include mesenteric lymph nodes.

Role of Estrogen in the Selective Transport of IgG1 into the Mammary Gland

K. L. SMITH

Ohio Agricultural Research and Development Center, Wooster 44691

The predominant immunoglobulin of bovine colostrum has been shown to be IgG1 and the work of Larson (18), Dixon (10) and Murphy et al. (23) indicates that IgG1 is selectively transported from blood serum to lacteal fluid before parturitions. Erb et al. (13) reported on the daily urinary estrogen excretion rate throughout pregnancy and showed that the excretion rate of estrogen increases greatly during the last 4 to 6 weeks of gestation. It was noted that this period of increasing estrogen coincides with the period of active colostrum formation in the bovine.

Experiments were undertaken to determine if the formation of normal colostrum, in large volume, could be achieved by injecting estrogen or estrogen plus progesterone into nonlactating dairy cows. A 4×4 Latin square design was used to determine the effect of four hormone treatments, consisting of a) estrogen (0.1 mg/kg/day); b) progesterone (0.25 mg/kg/day); c) estrogen plus progesterone; d) control (no hormone). There was approximately one month between treatments. Hormones were injected two times daily for seven days. Approximately 17 days after the last injection, the fluid present in the gland was removed and whey samples prepared. Total whey protein and the relative percentage of the individual whey proteins was determined. The G-200 7 S proteins, isolated from whey on Sephadex G-200, were subsequently resolved into IgG1, IgG2 and lactoferrin (Lf) by DEAE Sephadex A-50 chromatography. Mean amount of secretion removed following each of the four treatments was: control, 669.2 g; estrogen + progesterone, 4,023.4 g; estrogen, 1,385.3 g; and progesterone, 1,027.5 g. G-200 7 S protein on DEAE Sephadex A-50 is resolved into three distinct peaks. Peak I contains IgG2, Peak II contains Lf + IgG2, and Peak III contains IgG1. The distribution of G-200 7 S protein in DEAE Peaks I, II, and III respectively, following each of the treatments, was: control, 3.2, 39.2 and 57.6%; estrogen + progesterone, 2.7, 6.5 and 90.8%; estrogen, 5.8, 16.4 and 77.9%; progesterone, 3.3, 26.8 and 69.9%. The normal distribution for blood

serum G-200 7 S protein is 20, 20 and 60% and for fully involuted bovine mammary glands 2.6, 56.8 and 40.6%.

Glands filled with a colostral-like secretion after three of the four estrogen + progesterone treatments and one of the four estrogen treatments, whereas progesterone or control treatments never gave the response.

Conclusions are that the formation of normal colostrum can be achieved by injecting either estrogen or estrogen + progesterone. Chance of success appears to be greater if combination estrogen + progesterone is used. The results suggest that the hormones estrogen and progesterone are involved in the control of selective transport of IgG1 from blood serum to lacteal fluid.

Maternal Transport of Immunoglobulins to the Calf

T. E. Staley, E. W. Jones, and L. J. Bush
Oklahoma State University, Stillwater 74074

The transfer of immunoglobulins (Ig) from the serum of the dam, via the colostrum, to the serum of the calf was studied. Specific Ig (IgM and IgG) as well as total Ig were determined in the dams' sera and colostrums at parturition and in the calves' sera following suckling. Initially, calves were allowed to nurse ad libitum for the first day, then fed 4 liters of colostrum per day. Quantitative results on the transfer of colostral IgM and IgG showed that: a) colostral IgM and IgG were absorbed with equal facility, and b) peak concentrations for IgM and IgG in calf serum were 49 and 60% of the colostral level.

In a subsequent study, when calves were fed colostrum daily at 2.5 and 3.75% of body weight during the first and second days, total Ig transported produced the following results: a) blood sera in calves were significantly lower than in their respective dams, b) peak Ig concentrations in the sera of calves occurred 24 hr after birth, c) there was a positive linear correlation between colostral Ig and serum Ig concentrations attained in calves, and d) there was a positive relationship between the maximum Ig concentration in colostrum (at parturition) and the maximum Ig concentration attained in sera of calves after ingestion of colostrum.

Twenty-six and 30% of the calves in these studies remained hypogammaglobulinemic in spite of adequate levels in the ingested colostrum.

Electron microscopic examinations of the intestinal absorptive cells of the newborn calf have shown a similarity to the intestinal epithelial cells of other newborn ungulates. The ultrastructural intracellular organelle responsible for the uptake of IgG-ferritin conjugate, in both the jejunum and the ileum, is the tubular system in the apical cytoplasm. IgG-ferritin conjugate is transported the full length of the cell within vacuoles and released at the basal cell membrane.

Synthesis of Immunoglobulins by Various Tissues of the Cow

JOHN E. BUTLER,[1] CHARLES A. KIDDY,[2] CHARLOTTE MAXWELL,[1] MARTHA B. HYLTON,[3] and RICHARD ASOFSKY [3]
[1] Eastern Marketing and Nutrition Research Division ARS, USDA, Washington, D.C. 20250
[2] Animal Science Research Division, ARS, USDA, Washington, D.C. 20250
[3] National Institutes of Health, Bethesda, Maryland 21700

Qualitative in vitro studies of bovine immunoglobulin synthesis were performed on a broad spectrum of tissues obtained from two lactating cows and a one-week-old calf. The method of Hochwald et al. (15), using uniformly labeled lysine in NIH Media 320, was employed. Specific antisera to bovine IgA and glycoprotein-a (Gpa) and polyvalent antisera to

bovine immunoglobulins, whey proteins and whole bovine sera were employed. Organ cultures synthesizing immunoglobulins fit into three general patterns: a) Those synthesizing primarily IgA; b) those synthesizing primarily IgG and IgM; and c) those synthesizing all three classes of immunoglobulins in relatively equal amounts. Bovine IgA was the principal immunoglobulin synthesized by the ileum duodenum, colon, lungs, nasal mucosa, oral pharyngeal mucosa, and by the parotid, lacrimal and thymus glands. Spleen and lymph nodes from a wide range of sources, synthesized primarily IgG and IgM. The uterus, vagina, and mammary gland synthesized IgG, IgA and IgM although synthesis in the mammary gland was less pronounced than in most of the other tissues. Because separate precipitin arcs were not obtained for secretory IgA and Gpa when tested with antiserum to Gpa, it was difficult to distinguish newly synthesized Gpa from newly synthesized secretory IgA. Despite this difficulty, it was apparent that the mammary gland synthesized large amounts of Gpa. In a few tissues, including some mammary cultures, intense labeling of an anodal protein was obtained with antiserum to Gpa. This might represent the binding of Gpa to a protein in secretions other than IgA.

IgA-producing cells and hence an IgA-mediated local immune response in the bovine udder either did not develop or are disappearing as compared to nonruminant mammals. Excess Gpa synthesis in the bovine udder may be an evolutionary vestige or this protein may have some as yet unknown function in milk. The presence of a strong IgA-mediated immune system may be incompatible with the physiology of an organ secreting large amounts of protein over an extended period of time as is the case with the udder of domestic cattle. The importance of the bovine mammary gland in the production of IgG and IgM, and its function in the transport of these proteins from serum still requires study. IgA synthesis in the thymus gland may reflect the embryological origin of this organ from pharyngeal pouches.

The pattern of immunoglobulin synthesis in the calf paralleled that of the adult animals except for the lack of IgA synthesis in the calf gut and thymus. Proper absorption of maternal immunoglobulins by the calf gut may be correlated with the latent development of IgA in the gut.

With exception of the mammary gland, the data support the concept of an IgA-mediated secretory immune system in cattle similar to that described for nonruminant animals.

Fluorescent Localization of Immunoglobulins in the Tissues of the Cow

A. M. YURCHAK,[1] J. E. BUTLER,[2] and T. B. TOMASI, JR.[1]
[1] State University of New York at Buffalo, Buffalo 14200
[2] Eastern Marketing and Nutrition Research Division ARS, USDA, Washington, D.C. 20250

Previous fluorescent antibody studies in other species have shown striking differences in the relative numbers of IgG and IgA producing plasma cells in different tissues. IgG producing cells have been the predominant ones seen in the spleen and lymph node tissues and IgA cells have predominated in mammary, lung, and intestinal tissues. Selected tissues from six cows and two newborn calves were examined with a direct fluorescent antibody technique. Tissues were fixed in buffered formalin, rinsed in 30% sucrose overnight and sectioned on a cryostat. Sections were stained with fluorescein conjugated specific antisera to bovine IgG, IgA, IgM and glycoprotein-a (Gpa). Antisera to human IgG, IgA, IgM, and IgE and human secretory component were found to cross-react with their bovine counterparts and were found useful in confirming results with anti-bovine antisera. After staining with the fluorescein conjugates, all tissues were counter-stained with a rhodamine-normal rabbit serum conjugate to mask the nonspecific fluorescent staining of eosinophils often found in these tissues. The results of these studies are given in Table 1.

Staining of free IgA was found in the lamina propria of the intestine, between the epithelial

TABLE 1. Fluorescent immunoglobulins in tissues of the cow and new-born calf.

	IgG	IgA	IgM	IgE	Gpa
Small intestine	+++	++++	++	++	+++
Colon	+++	++++	++	ND	+++
Mammary gland	++	+	+	ND	+++
Submaxillary gland	+	+	−	ND	−
Spleen	++++	+	++	ND	±
Gut lymph nodes	++++	++	+++	ND	−
Lung lymph nodes	++++	+	+	ND	−
Femoral lymph nodes	++++	±	+	ND	−
Thymus	+	±	±	ND	±
Newborn calf	±	±	−	ND	±

ND, not determined.

cells and in the lumen of the crypts much as it has been found in other species. Fewer IgG producing cells were noted in the lamina propria but interstitial staining for free IgG was strong. Little luminal staining was found. Gpa was found intercellularly, in the lumen, and within the epithelial cells. Mammary tissues showed mainly IgG cells although cells producing other immunoglobulins were present in small numbers. Much more striking staining for IgG and Gpa was noted in the lumen of the lacteal ducts than was seen either intercellularly or within the epithelial cells. Few plasma cells were seen in the salivary glands examined and no staining for Gpa was seen although saliva from the same animals contained Gpa. This finding is interpreted as due to the insensitivity of the fluorescent method for detecting proteins that are rapidly secreted. The spleen and lymph nodes showed mainly IgG cells although the gut lymph nodes also showed moderate numbers of IgM and IgA cells.

In summary, our findings in the cow match in many ways previous studies in other animals. The predominance of IgA in intestinal tissues points to the presence of a "secretory system" in this species. The Gpa probably corresponds to the secretory component found attached to IgA in other species. The mammary gland is unique in that it apparently both produces immunoglobulins locally and also transports large amounts of IgG1. Studies of the distribution of cells producing IgG1 and IgG2 and IgE are in progress.

Changes in Levels of Immunoglobulins in Serum and Other Body Fluids Immediately Before and After Parturition

CHARLES A. KIDDY,[1] ROSEMARY McCANN,[1] CHARLOTTE MAXWELL,[2] CHARLES ROCK,[2] CAROL PIERCE [2] and JOHN E. BUTLER [2]

[1] Animal Science Research Division, ARS, USDA, Beltsville, Maryland 20750
[2] Eastern Marketing and Nutrition Research Division ARS, USDA, Washington, D.C. 20250

Weekly samples of serum, tears, lacteal secretions, saliva and urine were obtained from six Holstein-Friesian cows six weeks before and four weeks after calving. Amounts of total IgG, IgG1 and IgA (mg/ml) were determined by the radial diffusion method and total protein by the biuret method. Means for serum and lacteal secretions are in Table 1. Differences in levels among weeks, cows, and quarters of the mammary gland between cows were studies by analysis of variance. Correlations between individual protein levels in various secretions were calculated.

In lacteal secretions, differences due to weeks and cows were highly significant for all immunoglobulins measured. Quarter samples taken on the same day varied widely within cows in the concentration of immunoglobulins measured. A marked drop in the mean of all immunoglobulins occurred at parturition. IgG

accounted for 85% of the total whey protein before calving but only 5% after calving. Six weeks before calving the concentration of IgG1 in lacteal secretions was 95% of the total IgG but decreased to 85% during the week before calving. Similarly, in a postpartum mastitic quarter, IgG1 was only one-half of the total IgG. Also, the increase in serum IgG after parturition could not be accounted for solely by the concomitant rise in IgG1. These data suggest that IgG2 may be important in the lacteal secretions in late gestation and in mastitis.

Differences in amounts of IgG, IgG1 and IgA in the serum were significant among weeks and cows. Saliva levels were significantly different among weeks for IgA and IgG but not for IgG1. Immunoglobulin levels in tears were slightly higher than in saliva. Variation among cows for all three proteins in tears was highly significant but there were no significant differences among weeks. In saliva and tears (where IgA predominates), only IgG1 values were reliable because total IgG values were biased by antibodies against L-chains present in the IgG antisera but lacking in the IgG1 antiserum.

Since absolute concentrations of immunoglobulins in tears, saliva, and lacteal secretions are affected by dilution, the ratio of IgG/IgA was calculated from means in each sample. This ratio was highest in serum and lowest (less than one) in tears and saliva. In lacteal secretions the IgG/IgA ratio decreased markedly after calving for all cows. The overall ratios before and after calving were 19.8 and 7.3. This change in ratio can best be explained by postulating a decrease in the rate of the reported selective transport of IgG1 from serum to milk (10) while assuming local synthesis of IgA. Increased serum level of IgG after calving supports this hypothesis. The fluctuation in IgG and total protein levels associated with calving agree with those of Blakesmore and Garner (5).

Correlations between total IgG and IgG1 were positive and highly significant in lacteal secretions (.95+), serum (.76), tears (.59) and saliva (.51). These results indicate that the total IgG concentration is affected strongly by the amounts of IgG1; particularly so in the lacteal secretions and serum and less so in other secretions. Correlations between total IgG and IgA were highly significant and positive in the lacteal secretions (.73), tears (.70) and saliva (.85), but negative in serum (−.60). The negative correlation results from the combined effect of higher IgA levels in serum be-

TABLE 1. Changes in levels of immunoglobulins in the serum and lacteal secretions of cows at parturition. Weekly means for six cows (mg/ml).

Body fluid	Entity measured	Weeks prepartum							Col.[c]	Weeks postpartum				
		6	5	4	3	2	1	X ± SE[a]		1	2	3	4	X ± SE[a]
Lacteal secretions	Total whey ptn.[b]	NC	NC	53.7	52.8	48.9	51.3	51.7 ± 1.4		12.7	11.6	12.3	12.3	12.3 ± 0.7
	Total IgG	42.6	39.6	42.3	42.1	31.5	26.4	37.5 ± 1.6	17.6	2.91	1.37	0.99	0.62	1.47 ± 0.5
	IgG1	40.8	36.4	39.0	37.3	26.1	22.8	33.7 ± 1.7	14.5	2.29	1.08	0.77	0.69	1.20 ± 0.6
	IgA	2.8	2.1	2.1	2.5	1.6	1.2	2.0 ± 1.4	1.2[d]	0.37	0.17	0.16	0.15	0.21 ± 0.05
Serum	Total serum ptn.	65.8	65.6	59.3	57.9	61.6	66.0	62.7 ± 1.6		58.9	61.5	64.8	NC	61.7 ± 1.3
	Total IgG	24.8	21.9	21.5	20.6	19.4	19.9	21.5 ± 0.8		23.7	26.5	29.1	31.0	27.6 ± 1.7
	IgG1	14.5	12.8	13.0	11.4	9.4	8.9	11.7 ± 1.2		10.1	13.1	15.6	16.3	13.8 ± 1.4
	IgA	0.15	0.14	0.10	0.11	0.10	0.10	0.12 ± 0.008		0.07	0.06	0.05	0.05	0.05 ± 0.01

[a] Mean plus or minus standard error. [b] Protein. [c] Colostrum. [d] Mean colostral IgA concentration for one animal was 3.9 mg/ml. Individual quarters had levels as high as 6.4 mg/ml.

fore calving (presumably secretory IgA from the udder) and higher IgG levels after calving when the selective transport mechanism has slowed. The correlations between serum and lacteal secretion and serum and saliva levels of IgG were negative and highly significant (−.43 and −.44). The serum-tears correlation was not significant (.07). This is strong evidence that the transport mechanism moving IgG from serum to lacteal secretions is also operative in the salivary glands but not in the lacrimal glands. The serum-lacteal (.51), serum-saliva (.41) and serum-tears (.40) correlations for IgA were highly significant. These correlations strongly support the concept of a secretory origin for serum IgA as determined by Vaerman in the dog (30). In some of the comparisons the analysis indicated significant differences among individual cow correlations. It will be necessary to determine the extent to which these differences are real before definite interpretation of the overall correlations can be made.

The observation that IgG1 is the most important urinary IgG globulin is consistent with its reported higher turnover rate (24).

Bovine Secretory Immune System

JEAN-PIERRE MACH and JEAN-JACQUES PAHUD
University of Lausanne, Switzerland

Bovine secretory IgA (SIgA) was first recognized as a minor component of colostrum when compared with the concentration of IgG present (7, 20). In saliva, lacrimal, nasal and gastrointestinal secretions, SIgA is the major immunoglobulin (21). The amount of SIgA in these secretions is similar to that which has been found in the human. Thus, the cow has a normal secretory immune system with the exception of the mammary gland, which has acquired a special function of transporting IgG from serum to colostrum or milk. This observation holds for the sheep and goat (25). It should be noted that these three species transfer passive immunity to their young through the colostrum. It is known that species which are able to transfer IgG to the fetus through the placenta (human, monkey, and rabbit), have colostrum containing mostly SIgA. Thus, a colrelation can be drawn between the mode of transfer of immunity to the young and the type of immunoglobulin present in colostrum (Table 1). These differences in the functions of the mammary gland and other secretory organs were confined in the cow by mammary and salivary tissues cultured with radiolabeled amino acids (21).

Another finding was that the free form of the secretory component (FSC), usually associated with SIgA, was rather abundant in milk (8 mg/100 ml). Thus, the cow with its large amounts of saliva, rich in SIgA, and its abundant milk, seems to be one of the highest producers of SIgA and FSC.

TABLE 1. Relation between the mode of transfer of passive immunity to the offspring and the type of immunoglobulin in colostrum.[a]

Species transferring IgG to the fetus through placenta:	Species giving passive immunity through colostrum:
Human Monkeys Rabbit	Ox Sheep Goat Swine
Mammary gland is normally secretory, synthesizing mostly SIgA which is supposedly resistant to digestive enzymes, and may be active only in the digestive tract.	Mammary gland has acquired a special function of transporting IgG selectively from serum to colostrum. This IgG, needed by the newborn, is absorbed through the gut during the first and second day of life.

[a] All the species have a fully developed "secretory immune system"; they differ only in the function of their mammary glands.

The FSC isolated from bovine milk is physicochemically very similar to human FSC. Both combine in vitro with human polymeric myeloma IgA. Furthermore, the human and bovine FSC can combine in vitro with the serum IgA of nine different mammalian species including: human, ox, sheep, goat, horse, dog, swine, guinea pig and mouse (19).

SESSION III. BOVINE IMMUNE RESPONSE TO SYSTEMIC INFECTIONS

Chairman: M. James Freeman, Purdue University, Lafayette, Indiana

Lymphocyte Depletion and Immune Response in Calves Utilizing Closed System Filtration

C. T. Fitts, C. D. Graber, J. Majeski, and R. Sharbaugh
Medical University of South Carolina, Charleston 29400

To obviate previous problems with infection in calves depleted of lymphocytes by open methods, two types of filters placed in a closed extracorporeal thoracic duct (TD) to venous shunt system were tried. Initially ceramic beads were placed in the column utilizing varying sizes from 50 to 500 μ in diameter. No combination of these beads resulted in anything other than minimal removal of lymphocytes and the smallest beads would not permit adequate flow rates. Crushed optical glass of known average size became available at this time and various combinations were used as a filler in the column with good success. At the present time, crushed optical glass of 420 to 500 μ pore size is alternated with a layer of 44 to 88 μ pore size with glass wool placed at either end. This filter which must be changed at 48 hr is inexpensive and efficient, approximately 99% lymphocytes being removed. With this filter we have reduced thoracic duct lymphocytes to less than 3,000 cells/ml^3 which represents the immunounresponsive threshold. The peripheral lymphocyte level does not appear to be as reliable an index to tolerogenesis as thoracic duct levels. This draining period varies from two to three weeks depending on the calves' initial thoracic duct lymphocyte count and the lymph flow rate of the animal. At this point the following immunologic propensities of the calf were ablated:

1. Primary response to antigen (*Salmonella typhi*) did not occur when the animal was inoculated as early as two days within the tolerogenic period. Agglutinins and hemagglutinins to O and H antigens were negative at 10 days after antigenization. Similarly secondary response using the same organism did not occur in the depleted animal who had previously received primary antigenization weeks before drainage had begun.

2. Loss of ability to respond to a delayed sensitivity eliciting agent (old tuberculin) occurred in the drained calf previously sensitized to tuberculo-protein suspended in Freund's adjuvant prior to drainage. Recovery of both humoral and cellular mechanisms occurred when the animal was permitted to restore TD cell levels above the 3,000 lymphocytes per millimeter.

3. Complement (CH50) levels in the drained calf were not altered over those obtained with normal animals. Levels of five to six HU were maintained after several weeks of drainage. Serum lysozyme was similarly unaltered by drainage. Lymph chemistries including pH, Na, total protein, K, and total lipid did not vary as a result of filtration nor did they vary from the responsive to nonresponsive state.

4. A comparison of immunoelectrophoretic patterns of calf lymph and serum showed presence of an unidentified precipitin band just below the antigen well in the serum when both lymph and serum were precipitated by rabbit anti bovine sera. Monovalent antisera to IgG1, IgG2, IgM and IgA, graciously supplied by John E. Butler, demonstrated presence of these globulins in both lymph and sera of normal and depleted calves by IEP techniques. Thoracic duct lymphocytes of normal and depleted calves were reacted with antisera to human lymphocyte antigens (HLA 1, 2, 3, 9 and LC 19, 25). Such cells gave bright fluorescence when a fluorochrome was used as stain suggesting antigenic similarities between bovine and human lymphocytes.

Immunological Response of Cows to Brucella Antigens

ROBERT JENNESS and ROBERT K. ANDERSON
University of Minnesota, St. Paul 55101

The immunological response of nonvaccinated brucellosis-free lactating dairy cows to various brucella antigens administered in variable dosages by two routes was studied. The antigens were viable *Brucella abortus* (strain 19), heat inactivated *B. abortus* and soluble antigen obtained from *B. abortus* by sonification and fractionation by centrifugation, filtration, and removal of a small fraction insoluble at pH 3.5. It contained RNA, DNA, and protein in roughly equal amounts. Antigens were administered either by subcutaneous injection in the neck region or by infusion into the right rear quarter of the udder. In the latter case antigens were dispersed in 10 ml of milk for infusion. Udder inflammation, verified by physical examination and by analysis of the milk for chloride, was minimal. Blood serum and milk were collected daily following inoculation.

Anti-brucella titers of the serum were by the standard tube agglutination test (STT) with and without treatment with 2-mercaptoethanol (ME). Brucella antibodies in milk were assessed by the Brucella Ring Test (BRT). For animals infused in the right rear quarter, milk from that quarter was titered separately by the BRT.

Five cows injected subcutaneously with about 16×10^9 cells of viable strain 19, three injected with 5 or 10 ml of a 20% suspension of killed cells, and 2 which received 50 mg of soluble antigen all exhibited a rise in serum titer beginning about the third day and peaking in about 2 weeks. Much of the early titer represented mercaptoethanol-sensitive antibodies. The ME-stable titers tended to peak a little later than total titers. Brucella Ring test titers of milk rose in some cases at about the same time as those of serum; in several cases no increase in BRT occurred.

Twelve cows which received infusion of 2.0×10^5 to 1.5×10^6 strain 19 cells in the right rear quarter exhibited rather consistent response patterns, higher titers being obtained with the larger doses. Titers rose in about 7 days in the serum, early antibodies were largely ME-sensitive, considerable BRT titers developed in the infused quarters and sometimes but not always, small BRT titers were found in milk from the noninfused quarters.

Six cows infused with 20 to 100 mg of soluble antigen in the right rear quarter developed serum titers (from about the third day) followed by a slower but very dramatic rise in BRT in milk from the infused quarter; no titer was acquired in the other quarters.

Brucella specific antibodies were isolated from sera and milk taken at times selected by STT and BRT titers. The procedure involved adsorption of the antibodies from blood serum or milk whey on cellular antigens, centrifuging down the cells, washing, and eluting with 5 M urea. Recoveries of titer were of the order of 25 to 50% of the titer in the serum or whey. Analysis of the isolated preparations by immunoelectrophoresis, fractionation on Sephadex G-200, and centrifugation in sucrose gradients revealed large proportions of IgM immunoglobulins in the early antibodies of both blood serum and milk. The other principal class was IgG1. No definitive evidence for the presence of significant amounts of the IgA class was obtained in any case.

Immune Response to Foot-and-Mouth Disease Virus

K. M. COWAN and G. G. WAGNER
Veterinary Sciences Research Division
ARS, USDA, Greenport, New York 11944

Cattle infected with foot-and-mouth disease virus produced antibodies with different physical-chemical characteristics and in a sequential manner similar to that described for other animal species exposed to different types of antigens. The various antibody types were produced in sufficient quantities so that immunodiffusion techniques could be used for their investigation.

The antibody type detected by the 7th day was 19S γ_1-globulin and persisted at a demonstrable level until about 30 days after the cattle

were infected. It was eluted from diethylaminoethyl (DEAE)-cellulose at high ionic strength and did not give evident complement-fixation reactions.

By the 14th day, antibodies of lower sedimentation coefficient (S-rate) were detected and could be separated on DEAE-cellulose into fractions where the antibody activity had different electrophoretic mobilities. The antibody-containing fraction first eluted contained only a 7 S component, and the antibody activity occurred in the γ_2-globulin region on immunoelectrophoretic analysis. This 7 S-γ_2 antibody persisted at readily demonstrable levels for at least 180 days. The 7 S-γ_2 antibody-containing fraction fixed guinea pig complement but not to high levels.

Following the elution of 7 S-γ_2 antibody from DEAE-cellulose, a series of fractions was collected where the antibody activity was progressively more rapidly migrating upon electrophoresis. Two or 3 different electrophoretic classes of antibodies were demonstrable. The S-rates of these antibody types were not established, but there was some indication that they have rates of less than 19 S. These fast-migrating antibodies were demonstrable at 14 days postinfection and had complement-fixing activity. Therefore, the differing biological activity of the various physical-chemical classes of antibodies was clearly evident in the case of complement-fixing ability. It was pointed out that this is also probably the situation with neutralizing activity. Using guinea pig antibodies as a model system, it was found that 25 to 30 molecules of IgG2 were required to neutralize a foot-and-mouth disease virus, whereas only 2 or 3 IgM molecules were required. Clearly, satisfactory evaluation of the immune response requires a quantitative estimation of the different physical-chemical classes of antibodies present and the determination of their biological activity at a molecular level.

Finally, the application of the reversed radial immunodiffusion procedure was described for the quantitative determination of immunoglobulins in serum or secretions. The reversed procedure is more sensitive than the conventional procedure for detecting materials occurring in low concentrations in fluids, and has the added advantage of utilizing much smaller quantities of specific antisera.

Immune Response to Anaplasma marginale

J. E. ROSE, T. E. AMERAULT, and T. O. ROBY
Veterinary Sciences Research Division
ARS, USDA, Beltsville, Maryland 20705

Plasma samples from seven bovine carriers of anaplasmosis were fractionated by high speed centrifugation (35,000 rpm for 18 hr) on sucrose density gradients (10 to 40%). The resulting 10 fractions from each plasma sample were tested for agglutinating activity in a card agglutination test (2).

No agglutinating activity was found in any of the individual fractions from four of the seven plasma samples, but activity was found in mixtures of fractions. Mixtures of inactive fractions from the lower half of the gradient (19 S) with inactive fractions from the upper half (7 S) were active.

The response in fractions from the remaining three plasmas was slightly different. Some of the single fractions from the lower half of the gradient were individually weakly active, but mixtures of upper and lower fractions were much more active.

SESSION IV. BOVINE IMMUNE RESPONSE TO LOCAL INFECTIONS

Chairman: R. G. Thomson, Ontario Veterinary College, Guelph, Canada

Immunization with Bovine Enteroviruses and Quantitative Studies on Bovine Colostral IgG

TORBJORN MOLL
Washington State University, Pullman 99163

In some of the earlier serological works dealing with bovine enterovirus (BEV) strain differentiation, it was noted by several investigators that these viruses were inhibited to a considerable degree by normal rabbit serums and those of several other species. In our laboratory, it was found that pooled serums from normal sheep, rabbits, cattle, pigs, dogs, chickens, and horses all inhibited our BEV strains to a considerable degree (in 1/10 dilution: chicken, 57, sheep, 81, calf, 100, and cattle, 100%). Inhibiting antibody was also found in various commercial fractions (Cohn) of serums. Serum from chickens and young lambs had the least inhibiting activity.

In a recent immunization study, four of our BEV strains were adapted to a mouse fibroblast cell line where they formed clear plaques under methocel overlay. Virus stocks containing 7.5×10^5 to 8.2×10^6 PFU/ml were used to immunize rabbits. Seventy-two rabbits were randomly divided into 4 groups and each immunized with BEV strain I, II, III and IV. The results of the immunization showed very slight increases in homologous titers. When each serum was tested against all 4 BEV strains, there were no differences between homologous and heterologous titers. It was found subsequently that the mouse cell adapted viruses could not be adapted to rabbit cell cultures, and it was postulated that the lack of immune response in rabbits was due to the high preimmunization titers and failure of these viruses to multiply in rabbit cells.

For quantitative studies of IgG, serum and colostrum samples were collected from selected herds of dairy cattle during the months November through March. Blood and colostrum from all four quarters were collected soon after calving. The IgG contents of these samples were determined by the radial immunoprecipitation procedure (Table 1). The values obtained for total IgG concentrations were in reasonably good agreement with those obtained by other investigators.

It was found that a few serum samples and most colostrum samples gave double rings in the test. In such serum samples the outer ring was only slightly larger than the inner ring. In most colostrum samples the outer ring was considerably larger than the inner ring (Table 1). Klaus et al. (17) also observed double precipitation rings in the radial diffusion test. They gave no explanation for this and used the diameter of the outer ring for calculation of the total IgG concentration. Previous studies have indicated that the IgG1 and IgG2 concentrations in bovine serum are approximately equal but that the IgG1 is considerably higher in colostrum than the IgG2 concentration. To explore the double ring phenomenon further, a serum sample and a colostrum sample, showing double rings, were polyethylene glycol precipitated. The precipitates were redissolved in 0.02 M sodium phosphate buffer pH 6.5, and subjected to anion exchange chromatography (Sephadex A-50). Sample peaks eluted with 0.02 M NaCl, 0.15 M NaCl and 2.0 M NaCl were collected, concentrated to original volume, and tested in the radial immunodiffusion test. Each peak gave a single ring. The serum and colostrum samples as well as the anion exchange peaks were also tested on Ouchterlony plates. The serum and colostrum samples both gave double lines against anti-IgG whereas the peaks from anion exchange gave single lines.

From these preliminary studies we believe that the double ring phenomenon results from the presence of unshared antigenic determinants on IgG1 and IgG2. The data in Table 1 may not be accurate owing to the failure to use purified IgG1 and IgG2 preparations to establish a standard curve.

TABLE 1. IgG concentration in bovine colostrum and serum.

Group tested	Colostrum (mg/ml)						Serum (mg/ml)		
	Inner ring			Outer ring			Total IgG		
	Number samples	$\bar{X} \pm$ SE	Range	$\bar{X} \pm$ SE	Range		Number samples	$\bar{X} \pm$ SE	Range
Holstein	43	5.8 ± 4.4	0.7 — 16.2	52.2 ± 29.7	6.8 — 118.0		5[a]	14.5 ± 6.3	6.9 — 22
Guernsey	6	5.8 ± 0.6	3.1 — 8.0	95.3 ± 13.9	80.0 — 120.0		6	17.8 ± 2.6	13.4 — 20
Jersey	8	3.6 ± 2.1	1.8 — 8.0	71.5 ± 26.7	38.0 — 118.0		7[b]	10.5 ± 4.1	5.0 — 18
All samples	57	5.6 ± 4.1	0.7 — 16.2	51.4 ± 29.9	6.7 — 118.0		18	14.0 ± 4.0	5.0 — 22
Reported values:									
Ayrshires[c]	20			34.1 ± 2.1	11.5 — 46.2		100	12.9 ± 0.6	3.4 — 28.5
Mixed herd[d]	10			43.3 ± 14.0	26.3 — 70.7		100	26.4 ± 12.6	16.7 — 57.6

[a] One cow had an inner ring of 12.5 and an outer ring of 35.0.
[b] One cow had an inner ring of 5 and an outer ring of 10.8.
[c] Penhale and Christie (26).
[d] Klaus et al. (17).

Immune Response to Mammary Gland Infections

N. L. NORCROSS

New York State Veterinary College
Ithaca, New York 14850

The immunoglobulins in lacteal secretions vary with the phase of lactation. Colostrum contains, quantitatively, predominantly IgG1 or fast IgG. IgA and IgM have also been found in comparatively high concentrations. As lactation progresses the total amount of all the immunoglobulins decreases sharply. The amounts of the various immunoglobulins in milk have not been established quantitatively, however, the total amount has been found to be roughly less than 10% of that found in colostrum.

Most immunoglobulin in early lacteal secretions is IgG1 and presumably is derived from the serum. In addition, there is much evidence of the local production of immunoglobulins in the mammary gland. Reports of the local stimulation of specific immunoglobulins against many antigens by infusion of the latter into one or more quarters of the gland are found in the literature. Evidence for local production of immunoglobulins in the mammary gland stems from the increased presence of specific antibodies in the quarter that has been immunized as opposed to the other mammary quarters. It has been suggested with some preliminary evidence, that much of the locally produced antibody is of IgA class.

Mammary gland infections are, in large part, caused by species of staphylococci and streptococci, perhaps none of which contain antigens that stimulate the production of cross protective antibodies. It has been shown that antibodies against several of these pathogens can be detected in the milk after either systemic or local stimulation. The antibodies resulting from systemic immunization have not been demonstrated to be very effective in the milk. Inoculation of streptococcal vaccines into the area of the supra-mammary lymph node results in antibodies in the milk mainly of the IgG class, with some IgM and IgA antibodies also. Possibly these antibodies, like the anti-M protein antibodies of Group A streptococci in humans, render the streptococci susceptible to phagocytosis. When milk antibodies were tested using

the mouse protection test where there is an abundance of phagocytic cells, they were found protective. In the mammary gland, on the other hand, where phagocytosis is less efficient, less protective capacity was found.

The amount and protective capacity of the IgA antibodies produced by local infusion of antigen has not been determined.

Immune Response to Genital Infections

B. N. WILKIE, J. R. DUNCAN, and A. J. WINTER
New York State Veterinary College, Ithaca, New York 14850

Trichomoniasis, brucellosis, and vibriosis of cattle stimulate the production of cervico-vaginal (CVM) agglutinins which appear prior to or in the absence of serum antibody. Genital vibriosis is a venereal disease of cattle produced by *Vibrio fetus* venerealis and manifested by infertility, endometritis, and specific CVM antibodies. Histopathologically the disease results in focal sub-epithelial accumulations of lymphocytes and plasma cells within the reproductive tract. Component cells are highly pyroninophilic and blast transformations are observed. Immunofluorescence and electron microscopy have been used to identify the causative organism in epithelial cells and sub-epithelial mesenchymal cells of infected organs.

Five weeks following cervico-vaginal inoculation with suspensions of virulent *V. fetus* cells mucus contains anti-O agglutinins of the IgA type. Subcutaneous inoculation of killed whole *V. fetus* cells in Freund's complete adjuvant stimulates an increase in serum antibodies of both IgG and IgM classes as well as the appearance of anti-whole cell CVM agglutinins associated with the IgG class of immunoglobulins. Active immunization with either enteric vibrio (*V. fetus* intestinalis) or with venereal vibrio (*V. fetus* venerealis) results in the appearance of CVM agglutinins reactive with either strain but with a higher titer for the immunizing organism. Passive transfer of serum from immunized animals to normal non-immunized recipients results in the appearance of anti-whole cell CVM agglutinins of the IgG class. Actively immunized animals resist infection and colonization of the reproductive tract by virulent homologous *V. fetus*.

It would appear that transfer of IgG from serum to CVM can occur in cattle since passive immunization results in appearance of CVM IgG with anti-whole cell specificity, like that of the transfused serum. The possibility of local synthesis of IgG in the reproductive tract of actively immunized animals is not excluded. Local infection with *V. fetus* venerealis, on the contrary, results in predominantly IgG immunoglobulins of anti-O specificity. The reason for the different specificity has not been established.

Bovine Immune Response to Respiratory Infections of Viral Etiology

J. D. TODD
Jensen-Salsbery Laboratories, Kansas City, Missouri 64100

Calves 6 to 10 months of age which were devoid of neutralizing (SN) antibody against parainfluenza type 3 (PI3), infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD) viruses were exposed intranasally (IN) to these agents either alone or in combination to evaluate a) the time required for appearance of detectable SN antibody, b) the time of maximal titers, c) the correlation of serum antibody (S-Ab) with nasal secretion antibody (NS-Ab) levels at different times post-exposure and post-challenge, d) the correlation between antibody levels and virus excretions post-exposure and post-challenge, and e) the influence on antibody response of concurrent infection with virus combinations.

Eight calves were exposed to PI3 or PI3 and IBR. Antibody was present in serum from 1 of the 8 (1/8) on Day 7, 3/8 on Day 8, 6/8 on Day 9 and 8/8 on Day 10. Titers of S-Ab were maximal on Days 12 to 14 and were receding by post-exposure Day 21. Concurrent infection with IBR virus had no apparent effect on the time required for PI3 S-Ab to appear; however, maximal S-Ab titers to PI3 were 4-fold higher in calves with the combination infection than in calves infected with PI3 alone. NS-Ab against PI3 was present in all calves on Days 14, 17, and 21 in titers of 1:3 to $\geqq$ 1:12. These titers were not correlated with levels of S-Ab. Following challenge with PI3 virus, no secondary S-Ab response occurred, although titers increased slightly (2- to 4-fold) in some calves between post-challenge Days 7 and 14, and did not decrease by Day 21. Levels of NS-Ab were no higher 14 days post-challenge than maximal levels reached post-exposure, and by Day 21 had decreased 2- to 12-fold in most calves. Virus was recovered 7 to 9 days post-exposure from all calves and not at all post-challenge.

Twenty calves were exposed to IBR virus alone or in combination with PI3 or BVD (NY-1 strain) viruses. S-Ab against IBR was first detected in IBR or IBR-PI3 calves on post-exposure Day 8 (5 of 16), was present in 12/16 on Day 10 and in 16/16 on Day 12. Of four calves exposed to BVD virus 72 hr after exposure to IBR, only one had detectable S-Ab to IBR on Day 10, while 3/4 had S-Ab on Day 12, the remaining calf developing detectable S-Ab between Days 14 and 17. Maximal levels of S-Ab were present on Days 14 to 21, titers receding somewhat by Days 31 to 45. Concurrent infection with BVD virus apparently delayed the appearance of S-Ab against IBR and resulted in maximal titers 4-fold lower than those detected in sera of calves exposed only to IBR or to IBR and PI3 in combination. Concurrent infection with PI3 virus had no apparent effect on IBR S-Ab production. Detectable levels (1:3 to $\geqq$1:18) of NS-Ab were present in all of 9 calves (IBR or IBR-P13) tested on post-exposure Days 14, 17 and 21. Three of these calves were challenged on Day 31, resulting in elevations of NS-Ab by post-challenge Day 14 to $\geqq$1:12 while no increase in S-Ab occurred. Only 2 of the remaining 6 calves (not challenged) had detectable NS-Ab on Day 45 post-exposure, whereas S-Ab titers of 1:4 to 1:48 were still present. IBR virus was recovered from calves for 10 to 13 days post-exposure. Sporadic recoveries were made during the first 10 days post-challenge, indicating a low level of virus replication in some calves. Differences in NS-Ab and S-Ab neutralization kinetics were observed with both P13 and IBR post-exposure samples. NS-Ab appeared to be less avid than S-Ab, evidenced by partial neutralization of similar magnitude through several of the higher serial dilutions.

Interpretations of these results are summarized as follows: 1) Viruses which replicate extensively in cells of the respiratory tract epithelium evoke both humoral and secretory antibody responses. Persistence of NS-Ab is related to persistence or reapplication of the local stimulus. Viruses such as BVD which do not depend upon respiratory tract cells for primary replication do not induce the formation and elaboration of secretory antibodies. 2) The secretory and humoral antibody mechanisms are functionally independent as evidenced by a) lack of correlation between levels of S-Ab and NS-Ab, b) inverse relationships in increase or decrease of S-Ab and NS-Ab, c) the total absence of detectable NS-Ab in BVD infected calves which have high levels of S-Ab, and d) the differences observed in neutralization kinetics between S-Ab and NS-Ab against both IBR and PI3 viruses, suggesting lower avidity of NS-Ab.

Immune Response to Bacterial Respiratory Infections

J. R. DUNCAN, B. N. WILKIE, and A. J. WINTER
New York State Veterinary College, Ithaca 14850

Host resistance to respiratory tract disease resides in both nonspecific and specific defense mechanisms. Of the latter, most emphasis has been placed on the induction and assessment of local and humoral antibody as parameters of host resistance. It has been well established, particularly in man, that a secretory immune system (IgA) is of importance in respiratory tract infections and that its response is potentiated by local respiratory tract immunization.

Evidence for a secretory immune system in the bovine upper respiratory tract is now well established. The predominant immunoglobulin in bovine nasal secretions has a mol wt of approximately 400,000, a sedimentation coefficient of $11S_{20w}$ and a unique antigenic determinant not found in its serum equivalent. It may constitute up to 30% of the soluble protein in nasal secretions and has a 20-fold higher concentration on a total protein equivalent than in serum.

Earlier immunization studies with *Pasteurella hemolytica* clearly demonstrated that antibody production in the bovine respiratory tract is potentiated by aerosol in contrast to parenteral immunization and that the antibody activity in nasal secretions resides in an immunoglobulin class compatible with 11S IgA. The role of this antibody is colonization of the nasal passage by *P. hemolytica* and its protective functions in naturally occurring disease are not established conclusively.

A more recent study on natural antibody production to *Vibrio fetus* in nasal secretions corroborates the local nature of antibody production at various mucous surfaces. Concurrent determinations of total protein (TP), immunoglobulin (IgA, IgG1, and IgG2) and albumin levels in nasal secretion, tears and saliva has been made in order to define a possible index of local versus systemic origin of immunoglobulins in secretions of cattle. Results from 2 cattle suggest a definite correlation between a high albumin/(TP) ratio and a high IgG/TP ratio in nasal secretions while the IgA/TP ratio is independent of the albumin/TP ratio. The natural antibody titers to *V. fetus* were independent of the IgA/TP ratio or absolute level of IgA in nasal secretions while limited fractionation studies indicate all the natural antibody activity resides in the IgA fractions. Absolute values are given in Table 1.

TABLE 1. Total protein, albumin, and immunoglobulin levels in bovine nasal secretions.[a]

Animal	Protein	Weeks									
		1	2	3	4	5	6	7	8	9	10
		(mg/ml)									
26	Total	20.5	12.7	17.6	15.2	19.1	11.2	12.7	14.3	16.0	11.8
	Albumin	.13	.16	.10	.12	.13	.07	.10	.09	.12	.08
	IgA	3.25	1.98	2.53	1.78	1.98	.60	1.06	1.05	2.20	1.00
	IgG2	.06	.04	.07	.05	.03	.02	.02	.03	.03	.02
	IgG1	.19	.11	.16	.11	.10	.07	.07	.08	.10	.04
30	Total	17.6	13.8	16.4	15.2	21.0	9.3	8.8	15.4	13.9	
	Albumin	.07	.07	.28	.90	.46	.09	.16	.23	.16	
	IgA	1.50	1.23	2.25	1.30	2.14	.50	1.20	2.65	0.78	
	IgG2	.03	.02	.14	.49	.18	.03	.11	.23	.12	
	IgG1	.07	.17	.24	.73	.22	.03	.07	.28	.14	

[a] Total protein determined by biuret method. Albumin and immunoglobulin by radial immunodiffusion. IgM not determined.

SESSION V. HYPERSENSITIVITY

Chairman: R. M. Schwartzman, School of Veterinary Medicine, University of Pennsylvania, Philadelphia

Immediate Hypersensitivity—a Summary

S. G. CAMPBELL
New York State Veterinary College, Ithaca, New York 14850

A general review was made of the incidence, etiology, clinical findings and laboratory diagnosis of the clinical allergies which have been reported in cattle (8).

One specific condition, milk allergy, a naturally occurring syndrome in cattle was described as a model of a disease produced predominantly by hypersensitivity of the immediate type. This condition has an annual incidence of 0.5% in New York State and it is seen in most breeds of dairy cattle. Udder engorgement and actual milk let-down frequently precede an episode. Clinical signs of the disease are seen mostly in the respiratory system and the skin; they are due apparently to an increase in vascular permeability, increased secretions and smooth muscle contraction. These cows give a positive skin test with autologous, homologous but not heterologous milk at a dilution of 1:1,000. It is possible to transfer this hypersensitivity to calves passively with serum and to reproduce the syndrome experimentally by causing allergic cows to retain their own milk. It is therefore assumed that these cows become sensitized to milk or some component of it and when they are forced to retain milk they absorb some of it and show the manifestations of an immediate allergic reaction (9).

Finally it was pointed out that little definitive work has been done on the immediate form of hypersensitivity in cattle. Recent interest in the topic has revolved around its role in the pathogenesis of respiratory and gastro-intestinal disease in cattle. Relatively little definitive work has been done on the exact nature of the immunoglobulin responsible for the immediate form of hypersensitivity in cattle and most of the results to date are summarized in Table 1.

TABLE 1. Bovine reaginic antibodies.

	System used	Comment	Reference	Summary
Latent period of primary response	Ovalbumin, horse serum	8–10 days	f	7–10 days
	Ovalbumin	Most consistent results after 10 days	g	
	Ovalbumin	Minimum 7 days	a	
Immunoglobulin responsible	PCA in calves	Activity in IgG1 and IgG2 Only in IgG1 after 48 hr	d	Most persistent activity in IgG1
Direct skin test	Experimental model	Weal obvious, flare only on non-pigmented skin	e	Useful in natural and experimental conditions
	Natural allergy	Weals >20 mm considered positive	b	
Heat sensitivity	Experimental, ragweed	56 C, 2 hr (lost)	e	Heat labile (56 C)
	Experimental, ovalbumin	56 C, 4 hr (lost)	c	
	Natural, milk	56 C, 4 hr (lost)	b	
	Experimental, horse serum, ovalbumin	56 C, 30 min (reduced)	f	
Cold sensitivity	Experimental, horse serum, ovalbumin, PCA	−20 C Over a period of weeks	f	Possibly cold labile (−20 C)
Transfer of sensitivity to calves	Experimental models (skin transfer)	Successful with serum (0.1–0.2 ml)	PCA f PK c,e	Transfer of sensitivity successful using serum
	Experimental model, (systemic transfer)	Successful, various amounts serum	g	
	Natural allergy (skin transfer PK)	Successful with serum (0.1 ml)	b	
Transfer of sensitivity to guinea pigs	Guinea pigs, PCA	Heterologous transfer	f	Unsuccessful

[a] Aitken and Sanford (3).
[b] Campbell (9).
[c] Dungworth (11).
[d] Pierce (27).
[e] Weil and Reddin (31).
[f] Wells and Eyre (32).
[g] Wray and Thomlinson (33).

Delayed Hypersensitivity

JOSEPH QUINN
Ontario Veterinary College, Guelph, Canada

Delayed-type hypersensitivity is found to occur in individuals following infection with many bacterial, mycotic and viral agents. Microorganisms which induce delayed-type hypersensitivity appear to have one thing in common—they are intracellular parasites at some stage in infection. This allergic reaction can be elicited after infections such as tuberculosis, brucellosis, glanders, leprosy, Johne's disease, toxoplasmosis, and histoplasmosis. Among the viral agents which give rise to delayed-type hypersensitivity are vaccinia and mumps. While all viruses are obligate intracellular parasites, it appears that those which induce delayed-type hypersensitivity are primarily those which mature at the cell surface, such as vaccinia.

Delayed-type allergic reactions are placed in a special category for the principal reason that they cannot be shown to relate to circulating antibody. The procedure of passive transfer by serum is uniformly unsuccessful. Transfer by cells of lymphoid tissue, inflammatory exudates or white cells from the blood has been successful. The role of this allergic reaction in the area of cellular immunity to microbial infection is still not clear. Some findings suggest a correlation between modification of macrophages by intracellular parasites such as the tubercle bacillus and in vivo resistance to mycobacteria. However, other results do not support this concept. The relationship of immunological mechanisms to the clinical illness in Johne's disease have been examined and it is suggested by some workers that some of the lesions associated with Johne's disease in cattle may be due to the interaction of sensitized cells and antigen (delayed hypersensitivity) resulting in the release of cytotoxin and pyrogen which mediate the febrile responses, emaciation and anaemia.

With protein antigens, production of delayed-type hypersensitivity usually requires the use of Freund's adjuvant; contact sensitizers require percutaneous application. Tolerance can be produced by the introduction of appropriate materials by a route which produces wide dissemination but does not produce sensitization.

Manifestation of delayed-type hypersensitivity is often transient if no depot of antigen in the sensitizing form remains.

Migration Inhibition Test on Peripheral Bovine Leukocytes and Its Possible Application for the Diagnosis of Johne's Disease

OLE AALUND
Royal Veterinary and Agricultural University, Copenhagen, Denmark

The migration inhibition test on peripheral leukocytes has been found in a variety of systems to constitute a reliable in vitro procedure for the demonstration of delayed type hypersensitivity (1).

High yields of human leukocytes may be obtained by aspiration of the supernatant plasma, after sedimentation of the erythrocytes at 37C in a normal gravitational field ($1 \times g$). The erythrocytes from normal individuals of a variety of nonhuman species, including cattle and swine, have an extremely low sedimentation rate. In these species, the leukocytes were harvested from the buffy coat layer after centrifugation for 10 min at $1,000 \times g$ and the erythrocytes eliminated by hypotonic shock for 1 min in 1:45 Hanks' solution. This procedure proved equally applicable to rabbit and guinea pig blood.

The leukocyte yield averaged $39 \pm 12\%$ ($n = 56$) and only an insignificant change occurred in the differential count. Eighty to 90% of the harvested cells were viable as estimated by exclusion of trypan blue. Except for moderate changes in the appearance of the nuclei of the heterophils, the cell morphology was unaltered. Heparin, 25 IU per milliliter of blood, was preferred as the anticoagulant

since experiments with EDTA calcium depletion of the blood had demonstrated that the specific reactivity of the leukocytes was reduced to a period of six hours. The medium used was Difco TC 199 supplemented with 10% normal horse serum. The medium contained 500 IU penicillin and 500 mg streptomycin per milliliter.

The results from the testing of two Jersey herds with a low, but persistent incidence of cattle infected with *Mycobacterium paratuberculosis* are listed in Table 1. Animals were scored positive when their index of migration was more than 1 standard deviation below the mean index for the respective age group of the herd. The test antigens used were old type Johnin and avian tuberculin containing respectively 32 and 26 mg dry matter per milliliter.

None of the animals in Herd 1 reacted positively to both the leukocyte migration test and the complement fixation test. In Herd 2, only 2 animals were positive to both tests. The reaction appears to be specific since intradermal inoculation of the antigen removed the inhibition of migration for 2 weeks.

While these results are preliminary, they seem to indicate poor correlation between the leukocyte migration test and the complement fixation test. This lends hope to the possibility of a preclinical diagnosis of infections with *M. paratuberculosis* by the leukocyte migration test. The results also suggest that the leukocyte migration test probably can differentiate between Johnin and avian tuberculin hypersensitive individuals.

TABLE 1. Positive leukocyte migration inhibition tests in 2 Jersey herds infected with Johne's disease.

Age of the animals	Antigen and dose added to 1 ml migration chambers				Complement fixation tests
	Johnin		Avian tuberculin		
	0.20 ml	0.25 ml	0.05 ml	0.10 ml	
Herd 1					
≤2 yr	3/24[a]	9/24	1/24	4/24	0/24
>2 yr	2/52	8/52	4/48	3/52	2/52
Herd 2					
≤2 yr	4/13	1/12	4/10	3/12	2/13
>2 yr	8/75	11/76	4/36	3/40	7/76

[a] The numerator is the number of animals positive, the denominator is the number of animals tested.

References

(1) Aalund, O. 1970. The migration test on circulating bovine leukocytes and its possible application in the diagnosis of Johne's disease. Acta Vet. Scand., 11: 331.

(2) Amerault, T. E., and T. O. Roby. 1968. A rapid card agglutination test for bovine anaplasmosis. J. Amer. Vet. Med. Ass., 153: 1828.

(3) Aitken, M. M., and J. Sanford. 1968. Experimentally induced anaphylaxis in cattle. Vet. Rec., 82: 418.

(4) Blakeslee, D., J. Rapacz, and J. E. Butler. 1971. Bovine immunoglobulin allotypes. Symposium: Bovine Immune System. J. Dairy Sci., 54: 1319.

(4a) Blakeslee, D. J., J. E. Butler, and W. H. Stone. 1971. Immunogenetics of two immunoglobulin allotypes in cattle. J. Immunol., In Press.

(5) Blakesmore, F., and R. J. Garner. 1956. The maternal transference of antibodies in the bovine. J. Comp. Pathol., 66: 287.

(6) Butler, J. E. 1969. Bovine immunoglobulins: A review. J. Dairy Sci., 52: 1895.

(6a) Butler, J. E. 1971. A review of the bovine immunoglobulins. Symposium: Bovine Immune System. J. Dairy Sci., 54: 1315.

(7) Butler, J. E., E. J. Coulson, and M. L. Groves. 1968. Identification of glycoprotein-a as a probable fragment of bovine IgA. Abstr., Federation Proc.. 27: 617.

(7a) Butler, J. E., M. L. Groves and E. J. Coulson. 1970. The identification of a secretory immunoglobulin in the cow that is antigenically related to glycoprotein-a. Abstr., Federation Proc., 29: 642.

(8) Campbell, S. G. 1970. Clinical allergies in cattle. Cornell Vet., 60: 240.

(9) Campbell, S. G. 1970. Milk allergy, an autoallergic disease in cattle. Cornell Vet., 60: 684.

(10) Dixon, F. J., W. O. Weigle, and J. J. Vazques. 1961. Metabolism and mammary secretions of serum proteins in the cow. J. Lab. Invest., 10: 216.

(11) Dungworth, D. L. 1965. The pulmonary response of sensitized cattle to aerosol administration of antigen. Proc. Sym. Acute Pulmonary Emphysema. Laramie, Wyoming.

(12) Eichmann, K., H. Lackland, L. Hood, and R. M. Krause. 1970. Induction of rabbit antibody with molecular uniformity after immunization with Group C streptococci. J. Exp. Med., 131: 207.

(13) Erb, R. E., R. D. Randel, T. N. Mellin, and V. L. Estergreen, Jr. 1968. Urinary estrogen excretion rates during pregnancy in the bovine. J. Dairy Sci., 51: 416.

(14) Groves, M. L., L. W. Adler, and J. E. Butler. 1970. Unpublished data.
(15) Hochwald, G. M., G. J. Thorbecke, and R. Asofsky. 1961. A new technique for the demonstration of the synthesis of individual serum proteins by tissues *in vitro*. J. Exp. Med., 114: 459.
(16) Kickhofen, B., D. K. Hammer, and D. Schell. 1968. Isolation and characterization of γG type immunoglobulins from bovine serum and colostrum. Hoppe-Seyler's Z. Physiol. Chem., 349: 1755.
(17) Klaus, G. G. B., A. Bennett, and E. W. Jones. 1969. A quantitative study of the transfer of colostral immunoglobulins to the newborn calf. Immunology, 16: 293.
(18) Larson, B. L., and D. C. Gillespie. 1957. Origin of the major specific proteins in milk. J. Biol. Chem., 227: 565.
(19) Mach, J. P. 1970. *In vitro* combination of human and bovine free secretory component with IgA of various species. Nature, 228: 1278.
(20) Mach, J. P., J. J. Pahud, and H. Isliker. 1969. IgA with "secretory piece" in bovine colostrum and saliva. Nature, 223: 952.
(21) Mach, J. P., and J. J. Pahud. 1971. Secretory IgA, a major immunoglobulin in most bovine external secretions. J. Immunol., 106: 552.
(22) Milstein, C. P., and A. Feinstein. 1968. Comparative studies of two types of bovine immunoglobulin G heavy chains. Biochem. J., 107: 559.
(23) Murphy, F. A., O. Aalund, J. W. Osebold, and E. J. Carroll. 1964. Gamma globulins of bovine lacteal secretions. Arch. Biochem. Biophys., 108: 230.
(24) Nansen, P. 1970. Metabolism of Bovine Immunoglobulin G. Thesis. Munksgaard, Copenhagen, Denmark.
(25) Pahud, J. J., and J. P. Mach. 1970. Identification of secretory IgA, free secretory piece and serum IgA in the ovine and caprine species. Immunochemistry, 7: 679.
(26) Penhale, W. J., and G. Christie. 1969. Quantitative studies on bovine immunoglobulins. I. Adult plasma and colostrum levels. Res. Vet. Sci., 10: 493.
(27) Pierce, A. E. 1967. Immunization of the young animal. Passive immunization, selective transfer of immune globulins through bovine colostrum. 18th World Vet. Congr., 1: 407, Paris.
(28) Pink, R., and C. Milstein. 1970. Structure and evolution of immunoglobulins. Progr. Biophys. Mol. Biol., 21: 209.
(29) Porter, P., and D. E. Noakes. 1970. Immunoglobulin IgA in bovine serum and external secretions. Biochim. Biophys. Acta, 214: 107.
(30) Vaerman, J. P. 1970. Studies on IgA Immunoglobulins in Man and Animals. Thesis. Sintal-Louvain, Belgium.
(31) Weil, A. J., and L. Reddin. 1943. Dermal supersensitivity, heat labile, and heat stable antibody against ragweed in cattle. J. Immunol., 47: 345.
(32) Wells, P. W., and P. Eyre. 1970. Homocytotropic antibody demonstrated by passive cutaneous anaphylaxis in calves. Vet. Rec., 87: 173.
(33) Wray, C., and J. R. Thomlinson. 1969. Anaphylaxis in calves and the development of gastrointestinal lesions. J. Pathol., 98: 61.